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Quantitative Organic Analysis via Functional Groups

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Preface

It is the purpose of this book to give a series of working methods for determining organic compounds by the functional groups on the molecule. Determination of an organic compound by functional group analysis is much to be preferred to determination by analysis for the elements. The functional group is more characteristic of the organic compound than are the elements and, therefore, the analysis is more meaningful when the functional group is determined. The determination of the elements can be used only when the sample is one hundred per cent pure compound or when the compound to be determined contains an element not present in any of its impurities.

This book was written with a view to its use by laboratory analysts who find themselves in need of a method of analysis for a certain type of compound. It eliminates the need for searching through the literature and for weeding out and testing procedures that show promise. Having myself searched through the literature to obtain analytical procedures, I decided to put my findings in a book that would save other analysts much tedious work. This book, however, is not a series of literature references. Although I have been careful to give all sources for the procedures described, I have kept literature references to a minimum. I have described only the working procedures and have not mentioned the procedures found to be too limited or to be unsatisfactory for other reasons.

My book may also be used as a textbook or a laboratory manual in college courses in quantitative organic analysis. I recognize the great need which exists in our colleges and universities for formal training in the field of quantitative organic analysis, and I know that very few schools offer this training. With the present emphasis on organic chemistry, chemists are needed who are trained to do analytical work with compounds of this type. It is my belief that quantitative organic analysis should be included in the regular quantitative analytical courses that are usually entirely inorganic.

I wish to express my thanks to my coworkers, Mrs. Fred Maisch, Miss Irene Kervenski, Messrs Gordon Hanna, John Garis, Jr.,

Joseph Fitts, and Robert Edsberg, for their very able assistance in the development of some of the procedures described in this book and for their patience in testing the procedures obtained from the literature. I also wish to thank Dr. A. A. Benedetti-Pichler, Dr. L. T. Hallett, and Mr. Carl Cline, all of whom read and commented on the manuscript.

SIDNEY SIGGIA

February, 1949

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Introduction

This book deals primarily with the chemical analysis of organic compounds based on the reactions of functional groups. Some consideration has been given to physical methods of quantitative analysis and to methods for chemical separation of mixtures so that the compound to be analyzed can be separated from interfering impurities.

There are some functional groups for which no satisfactory chemical method of analysis can be found. Among them are nitriles, isonitriles, and ethers in which the groups on the oxygen atom are larger than butyl (the methyl through butyl ethers can be determined by alkoxyl determination). Methods for individual nitriles were found, but they are too specific for inclusion in a textbook dealing with more general procedures. Physical methods of analysis, however, can be applied in these cases.

The general principle of all the procedures described in this book is the determination of an acid, base, oxidant, reductant, or gas consumed or liberated by the reaction of the sample with a reagent. On this basis the reactions used are very simple, and the apparatus required is for the most part the standard quantitative analytical equipment.

The procedures are so described that they may be applied directly from this book without reference to the original papers. All the procedures have been used by the author and were chosen to meet the following standards: general applicability, simplicity, accuracy, and precision. For the analysis of some functional groups there are so few methods that no choice existed. There may be better procedures for the determination of certain groups, but those mentioned in this book are the best and simplest that were found. Most of the procedures have been slightly modified by the author and his coworkers. The changes were in the nature of sample size, volumes of reagents used, choice of simpler equipment, reaction time, and solvents. These changes were included because, in the testing of these procedures, they were found to improve the method slightly in simplicity, general applicability, accuracy, and precision.

Whenever possible, comments are included on interferences,

comparison with the other methods described for the same type of compound, the extent of the applicability of the procedure, and the accuracy and precision. The accuracy and precision are, unless otherwise stated, quoted as the arithmetic average deviation from the mean obtained from a series of determinations. Also, the accuracy and precision are generally quoted as percentage deviation relative to the amount of determined component contained in the sample.

Reference is made to textbooks that go into other aspects of quantitative organic analysis. On the subject of analysis for the elements, these books are very thorough: J. Niederl and V. Niederl, *Micromethods of Quantitative Organic Analysis*, 2nd Ed., John Wiley and Sons, New York, 1942; and Wilfred Scott, *Standard Methods of Chemical Analysis* (5th Ed. edited by N. Howell Furman), pp. 2460–2547, D. Van Nostrand Co., New. York, 1939. These books mention a few methods for the analysis of functional groups, some of which are described in this book. The textbooks found to be excellent guides for the physical methods of analyses are mentioned in the sections of this book dealing with physical methods.

All procedures discussed in this book can be reduced to a micro scale, using the methods described by A. A. Benedetti-Pichler, *Introduction to the Microtechnique of Inorganic Analysis*, John Wiley and Sons, 1942. Dr. Benedetti-Pichler describes general methods of converting practically any macro procedure to a micro scale without changing the concentrations of the reagents. He uses micro burettes and micro titration vessels to deal with the micro sample. If these techniques are used, the analytical procedure can be followed just as described in the macro form.

I Hydroxyl Groups

The reactivity of the hydroxyl group varies, depending on the rest of the molecule to which it is attached. Hydroxyl groups on primary and secondary carbon atoms and phenolic hydroxyl groups can be determined by esterification. Most phenolic hydroxyl groups can be indirectly determined by bromination (see Bromination Procedures, p. 111) and by coupling with a standard solution of a diazonium compound (see Coupling Procedures, p. 115). Hydroxyl groups on tertiary carbons and 2,4,6-trisubstituted phenols are difficult to determine because they do not esterify readily. Tertiary hydroxyl groups may be determined by the active hydrogen method described on p. 41, but this method is reliable only to $\pm 5\%$. (The active hydrogen method serves well in determining the number of hydroxyl groups per molecule. The accuracy and precision of this procedure are high enough to differentiate between monohydroxy, dihydroxy, trihydroxy, etc., compounds.)

ESTERIFICATION PROCEDURES

A convenient method for the determination of hydroxyl groups is esterification with an organic acid. Direct use of an organic acid results in the liberation of water and the reaching of an equilibrium. Because reaching an equilibrium is undesirable for a quantitative procedure the acid anhydride or the acid chloride is employed to eliminate this condition. Acetic anhydride and phthalic anhydride are the commonly used esterification agents.

Acetic anhydride and phthalic anhydride have each some advantages over the other when used as esterification agents. Acetic anhydride reacts more rapidly, less is necessary for completeness of reaction, and its applicability is more general. For example, acetic anhydride reacts readily with 1,2-glycols whereas phthalic anhydride seldom reacts quantitatively when the hydroxyls are so arranged. Even when the hydroxyl groups are more widely separated, as in 2-butyne-1,4-diol ($HOCH_2C{\equiv}CCH_2OH$), phthalic anhydride reacts slowly, and at least 10 moles of anhydride per

mole of hydroxyl group are necessary for complete reaction. Acetic anhydride, however, reacts rapidly, and only 3 moles of anhydride per mole of hydroxyl in the butynediol are needed for quantitative results. Acetic anhydride will react quantitatively with most phenolic hydroxyl groups, whereas the phthalic anhydride will not react quantitatively if it will react at all.

Phthalic anhydride is less volatile than the acetic anhydride, and, therefore, there is less chance of loss when the phthalic is used. Also, the phthalic anhydride can be used to determine hydroxyl groups in the presence of low-molecular-weight aldehydes, whereas these aldehydes interfere when acetic anhydride is used.

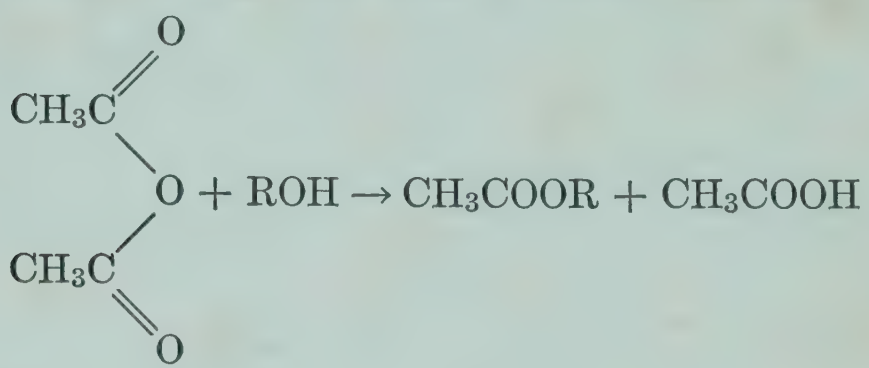
Pyridine is the medium employed for the esterifications.

For using acetic anhydride as the esterification agent, the method of Ogg, Porter, and Willits is the latest and has been found to be very reliable. The authors review the previous work done with acetic anhydride and they correct the difficulties encountered in those procedures.

ACETYLATION

Determination of Hydroxyl Content of Organic Compounds Adapted from the Acetic Anhydride Method of Ogg, Porter, and Willits

(*Ind. Eng. Chem.*, Anal. Ed., **17**, 394–397 [1945].)



Reagents.

Acetylating reagent consisting of one volume of A.C.S. grade acetic anhydride and three volumes of reagent pyridine. Reagent should be prepared fresh each day.

n-Butanol—technical grade.

Mixed indicator solution—one part of 0.1% aqueous cresol red neutralized with sodium hydroxide and three parts of 0.1% thymol blue neutralized with sodium hydroxide.

Standard alcoholic sodium hydroxide, approximately 0.5 *N*. Sodium hydroxide, 0.1 *N*, can be used for semi-micro samples, but the clarity of

the endpoint is poorer than when the 0.5 *N* reagent is used. Alcoholic sodium hydroxide is best prepared by mixing the required amount of saturated aqueous sodium hydroxide (approximately 18 *N*) with aldehyde-free ethanol or with c.p. methanol. The alcoholic alkali is standardized against potassium acid phthalate or against standard acid by use of the mixed indicator.

Procedure. A weighed sample containing about 0.005 to 0.008 mole of hydroxyl is introduced into a glass-stoppered iodine flask together with 5.00 ml of the acetic anhydride—pyridine reagent. The acetylating solution should be accurately measured, a pipette being used. The glass stopper should be well moistened with pyridine and loosely seated. The flask is put on a steam bath for 45 minutes. Then 10 ml of water is added by way of the well on the top of the flask, and the flask is swirled to bring the water in contact with all the reagent. After 2 minutes, the flask is cooled in ice or under running water, with the stopper partly open to prevent a partial vacuum from forming inside the flask. The sides of the flask and the stopper are rinsed with 10 ml of *n*-butanol, a few drops of indicator are added, and the contents are titrated with 0.5 *N* sodium hydroxide. If the sample contains 0.001 mole of hydroxyl, it is advisable to titrate with 0.1 *N* sodium hydroxide even though the endpoint may not be as sharp as with the 0.5 *N* reagent.

Samples which yield highly colored solutions making the indicator useless can be titrated potentiometrically by use of a potentiometer or *p*H meter with glass and calomel electrodes.

Any free acid or alkali in the sample should be determined on a separate sample by dissolving the sample in 5 ml of pyridine and titrating with standard alkali or acid, the mixed indicator being used.

Calculations.

Milliliters of NaOH used for blank − milliliters of NaOH used for sample (corrections being applied for any free acid or alkali which may be present) = *A*

$$\frac{A \times N\,\text{NaOH} \times \text{OH} \times 100}{\text{Grams sample} \times 1000} = \%\ \text{OH}$$

If the sample contains considerable amounts of water (more than 0.002 mole), additional acetic anhydride should be added so that enough will be present to acetylate the alcohol readily.

Water does not enter into the calculations, but it does destroy the reagent by hydrolyzing it to acetic acid. If too much reagent is destroyed there would not be enough present to esterify the alcohol completely.

Primary and secondary amines will interfere in this analysis. In fact, they acetylate so readily that this procedure can be used to determine them quantitatively (see Amino Groups).

Aldehydes of low molecular weight also interfere by reacting with the anhydride. The reaction between the anhydride and the aldehyde is not altogether clear. Some investigators blame the interference on condensation of the aldehyde to form hydroxyl-containing compounds, but this does not seem to be the case because some aldehydes which do not condense in this manner also interfere. In addition, analyses with phthalic anhydride are not affected by the presence of aldehydes, which would not be true if hydroxyl-containing compounds were formed. Formaldehyde interferes seriously, acetaldehyde less; the interference decreases with the higher aldehydes. No stoichiometric relationship between aldehyde and anhydride has been established.

It is well (keeping the equation on p. 4 in mind) to remember that when all the anhydride is consumed there will still be a titration equal to one-half the blank. If, when unknown samples are analyzed, the titration should be in the vicinity of one-half the blank, it is best to repeat the analysis with a smaller sample to insure having enough reagent for all the hydroxyl present.

Ogg, Porter, and Willits have successfully determined the following hydroxyl compounds: dihydroxystearic acid, monohydroxystearic acid, oleyl alcohol, cyclohexanol, and benzyl alcohol.

The author, in using the above procedure, has found it to work successfully for alcohols from methanol to octadecanol, ethylene glycol, glycerol, glycerol monoacetate, phenol, octylphenol, decyl- and dodecylphenol, ditertiary butylphenol, 2-butyne-1,4-diol, propargyl alcohol, and 3-methoxybutanol. With proper choice of sample size (0.005 to 0.008 mole of hydroxyl) an average accuracy and precision within 1.0% can be achieved.

As can be seen from the materials used to test the procedure, the procedure will not differentiate between hydroxyl groups on primary and secondary carbon atoms. Hydroxyl groups on tertiary carbon atoms and hydroxyls of 2,4,6-trisubstituted phenols do react with acetic anhydride but only very slightly.

This type of hydroxyl group cannot be determined by this method (there may be a few exceptions, but they are very few). Hydroxyl groups in less highly substituted phenols react readily with acetic anhydride, although these hydroxyl groups might be considered to be attached to tertiary carbon atoms.

PHTHALATION

As was mentioned above, aldehydes interfere with the acetic anhydride method. Therefore, when the hydroxyl group is determined in the presence of aldehydes, phthalic anhydride is more useful as an esterification agent than acetic anhydride. The phthalic anhydride reacts more slowly than the acetic anhydride, and a larger excess is needed for complete reaction. Also, phthalic anhydride will not react with phenols as does acetic anhydride so that alcohols can be determined in the presence of phenols.

Adapted from the Method of George Verbeck (Portland Cement Association)

(Presented in a talk before the Chicago Section of the American Chemical Society held January 24, 1947, at the Northwestern Technological Institute, Evanston, Illinois.)

Reagents.

1 *M* phthalic anhydride in pyridine.
1 *N* sodium hydroxide (aqueous).
Phenolphthalein indicator.

Procedure. A sample containing 0.02 mole of hydroxyl is placed in a 500-ml glass-stoppered Erlenmeyer flask together with 100 ml of 1 *M* phthalic anhydride in pyridine. The stopper is kept lubricated with pyridine while the flask is heated on a steam bath for 1 hour. After the heating, 10 ml water is added, and the flask is heated for 2 to 3 minutes longer. The flask is then removed, cooled to room temperature in ice, and titrated with 1 *N* standard sodium hydroxide, phenolphthalein being used. A blank is run on the phthalic anhydride reagent as well as on the sample.

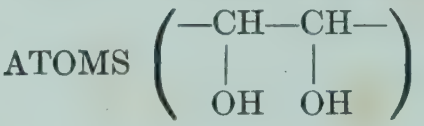
Some samples require a larger excess of phthalic anhydride than mentioned above. These samples are not common. 2-Butyne-1,4-diol, for instance, requires a tenfold excess of phthalic anhydride for quantitative esterification.

Calculations.

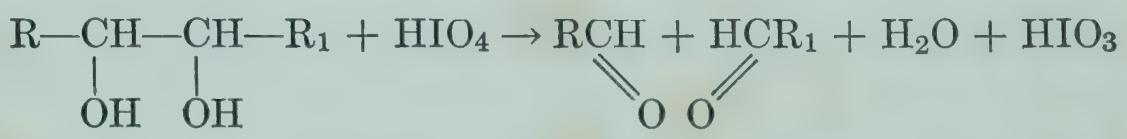
$$\frac{A \times N\ \text{NaOH} \times \text{mol. wt. of OH} \times 100}{\text{Grams sample} \times 1000} = \%\ \text{OH}$$

A = Milliliters for blank − milliliters for sample

HYDROXYL GROUPS ON ADJACENT CARBON ATOMS



Molecules containing hydroxyl groups attached to adjacent carbon atoms are readily oxidized by periodic acid. The reaction is quite specific and clean cut. Single hydroxyl groups or hydroxyl groups not attached to contiguous carbons are generally not attacked.



Adapted from the Method of W. D. Pohle, V. C. Mehlenbacher, and J. H. Cook

(*Oil and Soap*, **22**, 115–119 [May 1945].)

Reagents.

Standard 0.1 N sodium thiosulfate.

Oxidizing reagent: To a solution of 5 g of periodic acid (HIO_4) in 200 ml of distilled water is added 800 ml of glacial acetic acid. The solution should be kept in a dark, well-stoppered bottle.

Potassium iodide solution. 200 g per liter.

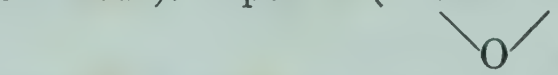
1% starch indicator solution.

Procedure. A sample containing approximately 0.0005 to 0.001 mole of dihydroxy compound is weighed into a glass-stoppered iodine flask. To the sample is added 100 ml of oxidizing reagent. A blank is run on the oxidizing agent alone. The solution is allowed to stand for one-half hour at room temperature. (For most compounds one-half hour is sufficient; however, a few samples require 1 hour for complete reaction.) Then 20 ml of potassium iodide solution is added, and the liberated iodine is titrated with 0.1 N sodium thiosulfate.

Calculations.

$$\text{Milliliters for blank} - \text{milliliters for sample} = A$$

$$\frac{A \times N \text{ thiosulfate} \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 2000} = \%\ \text{dihydroxy compound}$$

The procedure as described above was originated to determine various monoglycerides. However, it also works well for other dihydroxyl compounds such as ethylene glycol, mannitol, glycerol (consumes 2 moles of periodic acid per mole glycerol), dextrose, tartaric acid (needs to stand for 1 hour). Epoxide () compounds may also be determined by this method, but a longer time (one and one-half hours) and an eightfold excess of periodic acid are necessary.

The titration for the sample should be more than 80% of the blank to make sure that enough reagent is present for complete oxidation because the iodate formed in the reaction also liberates iodine from potassium iodide. If all the periodic acid were reacted, the titration would be 75% of the blank. Caution should be taken where the reaction products are formaldehyde or formic acid because these materials are subject to slow, though definite, oxidation at room temperature.

To analyze mixtures of glycerol and dihydroxy compounds this method can be used in combination with the method of Bradford, Pohle, Gunther, and Mehlenbacher.[1] The latter method involves periodic acid oxidation and titration of the formic acid formed by reaction with the glycerol. The method described here will yield both the glycerol and the dihydroxy compound. The difference between the results of both analyses will yield the dihydroxy compound.

In general, periodic acid will not oxidize such compounds as olefins, alcohols, and aldehydes. Some compounds not containing adjacent hydroxyls are attacked. An example is 2-butyne-1,4-diol ($HOCH_2C{\equiv}C{\cdot}CH_2OH$). It oxidizes very slowly, however, but significantly. Most amines are also attacked to some degree.

[1] *Oil and Soap*, **19**, 189 (1942).

2 Carbonyl Groups

There are two good methods of determining carbonyl compounds. In one method the bisulfite addition product is formed; in the other the oxime is formed by the use of hydroxylamine hydrochloride. The bisulfite procedure is rather specific for aldehydes. Only a few ketones can be determined by this method. The hydroxylamine procedure, being more general, will determine both aldehydes and ketones.

The bisulfite procedure involves use of excess bisulfite and the determination of the excess via an acid-base titration. This acidimetric procedure (procedure of Siggia and Maxcy on p. 13) has the advantages over other bisulfite methods of overcoming equilibrium difficulties and using more stable reagents.

DETERMINATION OF ALDEHYDES AND SOME KETONES BY THE BISULFITE METHOD

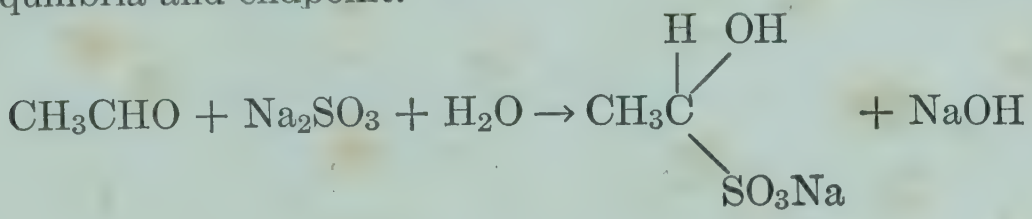
That aldehydes add bisulfite has been known for many years and has been used as the basis for some analytical methods for the determination of aldehydes. Parkinson and Wagner's paper[1] sums up the iodimetric methods for determining excess bisulfite and also gives methods for overcoming some of the difficulties in the earlier procedures.

The iodimetric methods require standardization of the bisulfite solution each day because of the instability of the solution. Also, difficulties with the equilibrium between aldehyde and bisulfite versus aldehyde bisulfite addition product sometimes affect results. As the iodine consumes excess bisulfite, the addition product dissociates back to free aldehyde and bisulfite. The extent of the error depends on the equilibrium constant for the particular aldehyde, amount of bisulfite addition product present, amount of iodine present, and the time it is in the solution.

The determination of increase in alkalinity in the system where aldehydes react with sodium sulfite has been used to determine

[1] A. Parkinson and E. Wagner, *Ind. Eng. Chem.* (Anal. Ed.), **6**, 433–436 (1934).

aldehydes.[2] However, the existing methods exhibit difficulties of equilibria and endpoint.



There is an equilibrium involved in the above reaction, and the reverse reaction is quite pronounced. In the determination of acetaldehyde, because of its low boiling point, much aldehyde is lost from the solution. Seyewetz and Bardin tried to overcome this difficulty by keeping the acetaldehyde content in their samples down to 7 to 8%. Also, they chilled the solutions to 4 to 5°C.

In attempts to use the Seyewetz and Bardin procedure, the disadvantage mentioned was very evident. The equilibrium caused trouble with the low-boiling aldehydes because of consistent loss of aldehyde and also caused trouble with the higher boiling aldehydes because of the insolubility of the free aldehyde. The procedure can be used for determining formaldehyde (Seyewetz and Gibello[3]) because of its relatively high solubility. A formaldehyde procedure of this type is also described by D'Alelio.[4]

Romeo and D'Amico,[5] realizing the equilibrium difficulties in the previous procedures, used sodium sulfite–potassum bisulfite mixtures to determine cinnamaldehyde and benzaldehyde, mentioning good results for cinnamaldehyde but poor results for benzaldehyde. They tried the method on some ketones, but results were generally poor.

The function of the bisulfite is to shift the equilibrium which occurs when sulfite alone is used. However, because of the instability of bisulfite solutions, it was found advisable in our work to use sulfuric acid instead. An aliquot of standard sulfuric acid is added to a large excess of sodium sulfite solution to produce sodium bisulfite *in situ*. The acid is very stable and can stand for long periods of time without the titer changing significantly. The acid is not added to the sulfite until just before the aldehyde

[2] Seyewetz and Bardin, *Bull. soc. chim.* (3), **33,** 1000–1002 (1905).

[3] Seyewetz and Gibello, *Bull. soc. chim.* (3), **31,** 691 (1904).

[4] G. F. D'Alelio, *Experimental Plastics and Synthetic Resins*, p. 164, John Wiley and Sons, New York, 1946.

[5] G. Romeo and E. D'Amico, *Ann. chim. applicata*, **15,** 320–330 (1925).

sample is introduced. The aldehyde reacts with the bisulfite, and the excess bisulfite is titrated with standard alkali. (The reaction might also be viewed as the aldehyde reacting with sulfite to liberate sodium hydroxide and the acid present consuming the sodium hydroxide, forcing the reaction to completion.) The large excess of sulfite is to keep the reaction essentially at completion as the excess bisulfite is titrated with alkali. In this system, the reaction is so near completion that no aldehyde can be detected above the solution, even in the case of low-boiling aldehydes such as acetaldehyde. This reagent also dissolves many insoluble, high-boiling aldehydes.

To detect the endpoint more accurately, it was found advisable to use a *p*H meter in the titration. A curve is plotted of *p*H

Table 1

Aldehyde	*p*H *Range of Solution at Endpoint*	*Observed Value (Moles)*	*Moles Sample Taken*
Acetaldehyde	9.05–9.15	0.02455	0.02458
		0.02120	0.02118
		0.02858 (*p*H 9.1)*	0.02862
Propionaldehyde	9.30–9.50	0.02085	0.02090
		0.02037	0.02053
		0.01792 (*p*H 9.4)*	0.01811
Butyraldehyde	9.40–9.50	0.0249	0.0243
		0.0233	0.0236
		0.0275 (*p*H 9.45)*	0.0280
Cinnamaldehyde	9.50–9.60	0.0105	0.0106
(Takes on 2 moles bisulfite		0.0108	0.0110
per mole sample)		0.0120 (*p*H 9.55)*	0.0122
Crotonaldehyde	9.20–9.40	0.0250	0.0253
(Takes on 2 moles bisulfite		0.0189	0.0190
per mole sample)		0.0235 (*p*H 9.30)*	0.0238
Benzaldehyde	8.85–9.05	0.0163	0.0164
		0.0168	0.0173
		0.0147 (*p*H 8.95)*	0.0154

* Rapid method used.

Note. All samples were distilled in a Podbielniak fractionating column (manufacturer claims 100 plates) and analyzed less than 2 hours after distillation to minimize air and autoxidation of sample.

versus milliliters of standard alkali added, and the endpoint is determined from the curve. As a short cut, it can be noted that the endpoint in the case of each aldehyde comes at a rather definite *p*H (Table 1). (There is only slight deviation with the size of

sample.) Once this *p*H is known, each sample can be titrated to that *p*H, thus eliminating the necessity of plotting a curve for each sample.

The advantages of this procedure are that it is rather generally applicable to determining aldehydes and that it has overcome the difficulties of equilibrium, such as incomplete reaction, loss of sample, and poor titration. This procedure has also overcome the endpoint difficulties encountered in the previous procedures and has eliminated the necessity of using unstable standard solutions.

Adapted from the Method of Siggia and Maxcy

(*Ind. Eng. Chem.*, Anal. Ed., **19,** 1023 [1947].)

Reagents.

1 *M* sodium sulfite.
Standard 1 *N* NaOH.
Standard 1 *N* H_2SO_4.

Procedure. To 250 ml of 1 *M* sodium sulfite in a 500-ml glass-stoppered Erlenmeyer flask is added 50 ml of 1 *N* sulfuric acid. The flask is swirled as the acid is added, to prevent the loss of sulfur dioxide caused by localized overneutralization of the sodium sulfite. To this solution is added, sealed in a glass ampoule (see Section 25), a sample containing 0.02 to 0.04 mole aldehyde. The flask is then stoppered, the stopper being greased for low-boiling aldehydes to prevent any loss. It is then vigorously shaken to break the ampoule containing the sample. Some glass beads included in the flask with the ampoule will cause the ampoule to break more easily. The flask is then shaken for 2 to 3 minutes (5 minutes for the more insoluble aldehydes) to insure complete reaction. The contents are then quantitatively transferred to a beaker. Electrodes from a *p*H meter are inserted in the solution, and the solution is stirred. The *p*H of the solution is noted as standard 1 *N* alkali is added to titrate the excess acid.

For accurate results, the *p*H reading versus milliliters of alkali added are noted and plotted. The endpoint is determined from the plot. A more rapid method of determining the endpoint, though slightly less precise, is to add alkali until a *p*H is obtained corresponding to the *p*H at the endpoint for the particular aldehyde. This endpoint *p*H can either be taken from Table 1 or predetermined in cases of aldehydes not mentioned.

Sodium sulfite contains a small amount of free alkali as an impurity so that 250 ml of the solution consumes some acid. The blank is very small but not negligible; it amounts to about 0.4 to 0.5 ml of 1 *N* acid per 250 ml of sulfite solution. On each carboy of sodium sulfite solution prepared, the free alkali should be accounted for, or the aldehyde results will be slightly high. Rather than use a blank on the sulfite solution, it was found more satisfactory to add enough 1 *M* sodium bisulfite to the sodium sulfite to neutralize the free alkali and bring the *p*H of the sulfite to 9.1. This procedure eliminates the need of a blank and may be done only once to each carboy of solution.

Calculations.

A = Calculated amount of NaOH standard solution needed to titrate the 50 ml of standard acid used − the milliliters of standard NaOH used to titrate the sample

$$\frac{A \times N \text{ NaOH} \times \text{mol. wt. compound} \times 100}{\text{grams sample} \times 1000} = \% \text{ compound}$$

In the case of most of the aldehydes used above, the endpoint was sharp enough so that the rapid method of just titrating to the *p*H of the endpoint could cause an error in endpoint determination of only ±0.2 to 0.3 ml. This error is not very significant and can be nullified by using a rather large sample of about 0.04 mole of aldehyde, which consumes 40 ml of 1 *N* acid. It can readily be seen that a plot of *p*H versus milliliters of sodium hydroxide is not necessary once the *p*H at the endpoint of the particular aldehyde is determined. The reproducibility of the procedure is ±0.2% if the entire curve is plotted and ±0.4% if the rapid method is used.

The endpoint in the determination of benzaldehyde is relatively poor (see Fig. 1), and the rapid method could cause an error of ±0.4 to 0.5 ml. Here a plot is necessary to determine the endpoint accurately.

Ketones cannot, on the whole, be determined by this method, even though they do form bisulfite addition products. Acetone, methyl ethyl ketone, quinone, naphthoquinone, and cyclohexanone were tried. Cyclohexanone is the only ketone tried which might be determined by this method. The titration curves for ketones indicate that the ketone does react with bisulfite, but there

is no discernible endpoint (see Fig. 1). The *p*H climbs steadily on addition of the sodium hydroxide. In the determination of cyclohexanone, a poor endpoint is obtained comparable to that of benzaldehyde. From the titration curves in the determination of ketones, it seems that the equilibrium between ketone and ketone-bisulfite addition product leans toward free ketone and sodium bisulfite to a great extent. As sodium hydroxide is added, the *p*H rises gradually, but no break occurs in the curves. When

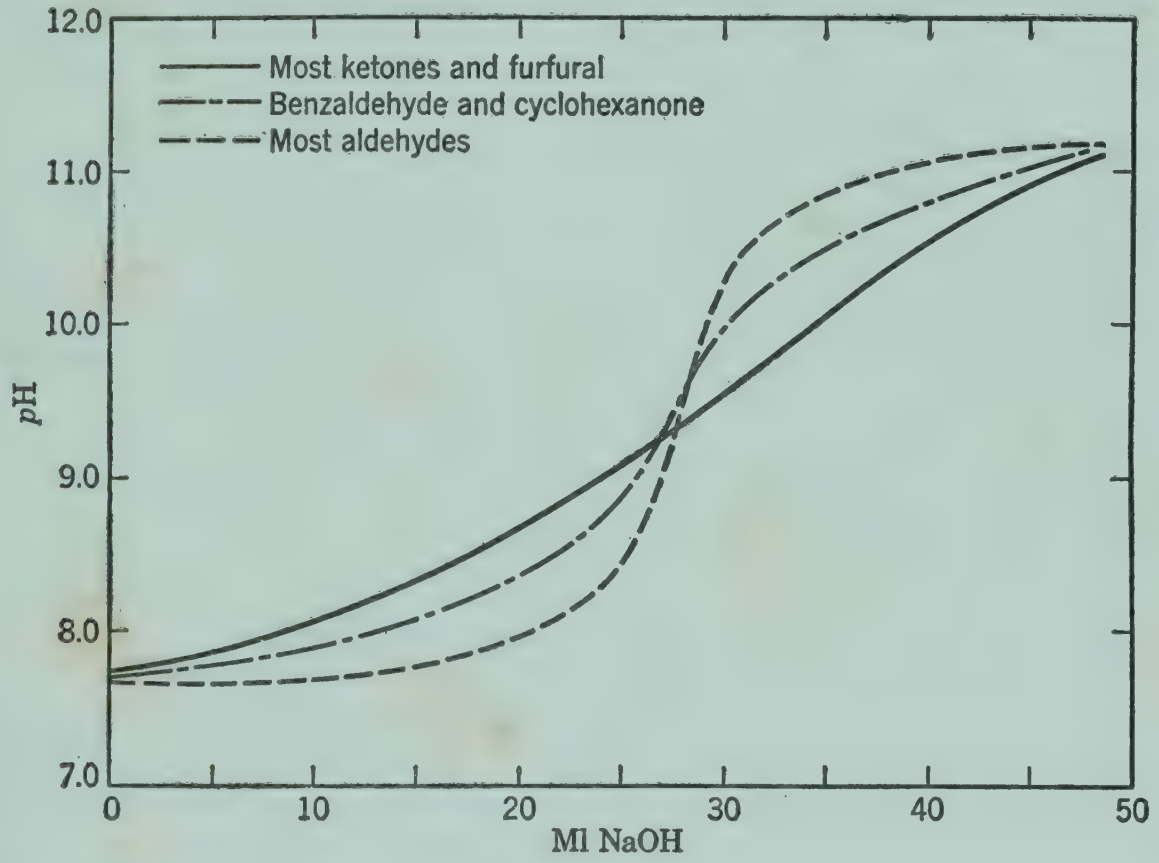


FIGURE 1.

excess sodium bisulfite is consumed by the sodium hydroxide the equilibrium is upset and some ketone bisulfite addition product is consumed, together with the excess sodium bisulfite, and no endpoints are obtained. This explanation can be applied in the determination of furfural, which behaves like the ketones.

The poor endpoint in the determination of benzaldehyde can be attributed to the same cause, but in this instance the equilibrium is enough in favor of the addition product to make this aldehyde determinable by this method.

Ketones, in general, will not interfere in the determination of aldehydes if they are not present in excess of about 10 mole %. The aldehyde produces a break in the *p*H versus milliliters of

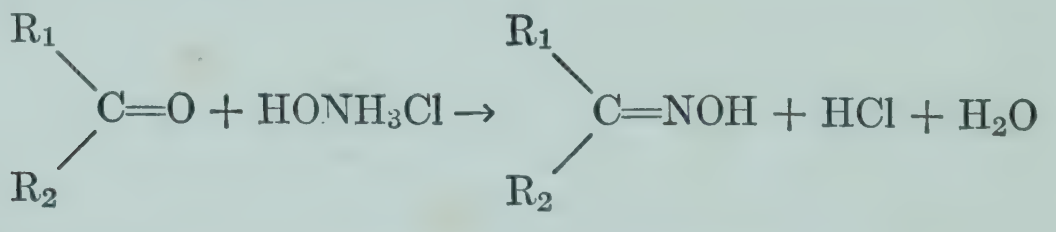
sodium hydroxide curve, whereas the ketone does not. If ketones are present, the entire curve of *p*H versus milliliters of sodium hydroxide has to be plotted and the break in the *p*H curve determined to obtain the endpoint. As is indicated by the graph (Fig. 1), the presence of ketones can affect the *p*H at the endpoint and, therefore, cause erroneous results if the rapid method of titrating to a definite *p*H value is used.

Acidic or basic impurities in the sample should be determined separately before the aldehyde procedure is applied, and the titration for the aldehyde should be corrected for the presence of these impurities.

Acetals will not interfere in the procedure. These compounds hydrolyze in strong acid solution to yield acetaldehyde. However, the *p*H of the sodium sulfite–sulfuric acid solution, in proportions described in the procedure, is about 6.8, and there is no noticeable hydrolysis of the acetals at this *p*H. Also, the aldehyde in the sample consumes the bisulfite so rapidly that the *p*H of the solution is raised to about 7.5 as soon as the sample comes in contact with the sodium sulfite–sulfuric acid solution, further lessening any possibility of hydrolysis.

HYDROXYLAMINE PROCEDURE FOR ALDEHYDES AND KETONES

The previous procedure is rather specific for aldehydes, being applicable for only one known ketone. The method described here is more general and will determine both types of carbonyl compounds. The method will not determine the carbonyl group of the carboxyl or amide type.



R_1 and R_2 can be hydrogen atoms.

Pyridine is used to displace the equilibrium existing in the above reaction by combining with the hydrochloric acid liberated. Pyridine hydrochloride is sufficiently acidic to be titrated with standard sodium hydroxide.

The rate of reaction between carbonyl compound and hydroxyl-

amine varies somewhat, depending on the groups attached to the carbonyl group. These hindrances, which are largely steric in nature, are seldom so great as to make the method inapplicable. Some aldehydes will react quantitatively in 30 minutes at room temperature; others may require 2 hours of heating at 98 to 100°C for complete reaction (see Table 2).

Adapted from the Method of W. M. D. Bryant and D. M. Smith

(*J. Am. Chem. Soc.*, **57**, 57–61 [1935].)

Reagents.

0.5 *N* hydroxylamine hydrochloride.

A solution is made containing 35 g of hydroxylamine hydrochloride dissolved in 160 ml of distilled water and diluted to 1 liter with 95% ethanol. The hydroxylamine hydrochloride should not contain excessive amounts of free acid. Ten grams of the hydrochloride in 50 ml of distilled water should not require more than 8 ml of 0.5 *N* sodium hydroxide to produce neutral (green) endpoint with bromophenol blue.

Pyridine-indicator solution: Twenty ml c.p. pyridine and 0.25 ml of a 4% bromophenol blue indicator diluted to a liter with 95% ethanol.

0.5 *N* sodium hydroxide prepared in 90% methanol. Methanol is used in preference to ethanol for the reason that methanol solutions do not darken on standing.

Procedure. Thirty ml of hydroxylamine hydrochloride reagent and 100 ml of pyridine-bromophenol blue solution are introduced into a 250 ml glass-stoppered Erlenmeyer flask, with reflux condenser to fit when heating is required. A 300-ml "Citrate of Magnesia" bottle can be used for low-boiling carbonyl compounds such as acetaldehyde. The sample is either weighed or pipetted into the reaction vessel in such amounts that about half the reagent is present after the reaction is complete. The flask is stoppered if no heat is required and is allowed to stand for the prescribed length of time. If the reaction mixture requires heat for the particular carbonyl compound being determined, the condenser is attached and the reaction flask is heated on a steam bath for the required time. The pressure bottles can also be heated on a steam bath for the required time, precautions being taken to have the bottle encircled with a piece of wire screening should the bottle contain a fault and burst on heating. The wire screening should not be greater than $\frac{1}{4}$-inch mesh. Heated samples should be cooled to within 5 degrees of room temperature before titration because the

TABLE 2. ANALYTICAL DATA FOR ALDEHYDES AND KETONES
(Figures in parentheses indicate number of determinations.)

Substance (Purity 98–100% except where noted)	*% of Theoretical Carbonyl Content* — *Cold Experiments* (30 min except where noted)	*Hot Experiments* (2 hr at 98–100°C except where noted)
Propionaldehyde		(3) 98.8 ± 2.0
Acetone	(3) 99.0 ± 0.7; (3) 99.1 ± 0.2 (5–10 min)	(2) 99.2 ± 1.1
Isobutyraldehyde	(2) 98.3 ± 0.3 (5–10 min)	(3) 99.0 ± 1.0; (2) 99.5 ± 0.0; (3) 98.5 ± 0.3
Pentanone-2	(2) 99.3 ± 0.5	(1) 100.0 (1 hr, 98–100°)
Pentanone-3	(2) 99.1 ± 0.2	
Hexanone-2	(2) 98.8 ± 0.3	
2-Methylpentanone-3	(1) 99.4 (27 hr)	(2) 100.5 ± 0.8; (2) 101.6 ± 0.4
2-Methylpentanone-4		(3) 100.0 ± 1.1
2,2-Dimethylbutanone-3	(2) 95.8 ± 0.0	(2) 98.4 ± 0.3 (1 hr, 98–100°)
2,4-Dimethylpentanone-3	(2) 99.9 ± 0.0 (48 hr)	(3) 94.9 ± 0.6*; (2) 95.5 ± 0.4*; (2) 100.8 ± 0.0 (stood 18 hr in cold after heating)
2-Methylhexen-(3)-one-5		(1) 101.5 (30 min, 98–100°) 102.5 ± 0.4 (1 hr, 98–100°)
2,4-Dimethylhexanone-3	(1) 98.4 (17 hr)	(2) 93.6 ± 0.4*; (2) 97.5 ± 0.5 (stood 20 hr in cold after heating)
Octanone-2	(2) 99.8 ± 0.0	(3) 98.7 ± 0.8
d-1-Camphor	(2) 93.5 ± 0.0 (34 days)	(2) 98.8 ± 0.2 (0.5-g sample, 5 hr, 98–100°)
Furfural	(2) 99.2 ± 0.6	
Benzaldehyde	(2) 101.6 ± 1.4	(3) 100.7 ± 2.0
p-Toluic aldehyde	(2) 101.0 ± 0.8	
Dimethylbenzaldehyde	(2) 101.8 ± 0.6	
Cinnamic aldehyde	(2) 98.3 ± 0.4	(2) 99.2 ± 0.2
Acetophenone	(1) 98.4 (3 hr)	(2) 100.2 ± 1.0
Salicylic aldehyde	(3) 98.0 ± 0.6 (2 days); (1) 96.4 (30 min)	(2) 98.0 ± 0.3 (1 hr, 98–100°)
o-Nitrobenzaldehyde	(1) 99.1	
p-Chlorobenzaldehyde	(1) 98.7 (10 min)	
Benzophenone		(1) 100.9 (3 hr, 98–100°)
Benzoin	(1) 98.5 (14 hr)	(2) 102.4 ± 1.1 (1 hr, 98–100°)
Benzil (basis of one reactive CO group)	(1) 100.4 (14 hr) (1) 101.8 (2 days)	(2) 105.2 ± 1.2 (1 hr, 98–100°)
2-Methylpenten-(2)-al-1†	(3) 98.2 ± 0.5; (2) 96.3 ± 0.7	(2) 97.6 ± 0.4
Heptanone-4†	(3) 95.1 ± 0.5	(2) 95.4 ± 0.7
2-Ethylexanal-1†	(3) 95.2 ± 0.7	
Acetylacetone†	(2) 95.5 ± 0.2 (2 days)	
Acetonylacetone†	(2) 97.2 ± 0.4	
Anhydrous glucose†	(1) 95.9 (43 hr)	(2) 96.3 ± 0.4; (2) 97.2 ± 01 (stood 69 hr in cold after heating)
Carvone†	(2) 95.2 ± 0.0 (24 hr)	(2) 95.5 ± 0.0

* Equilibrium for hot sample; goes to completion in cold.
† Purity better than 95%.

indicator color goes through a reversible change on heating and cooling. A blank should be run with each set of determinations for use as a color standard. The number of moles of sodium hydroxide used is equal to the number of moles of carbonyl compound.

To maintain the sensitive blue-green color of the blank, the volumetric ratio of reagent to pyridine should be kept constant. The color is also dependent on the water content of the final mixture. The presence of an appreciable amount of water in the sample plus that included in the reagent, increases the alkaline buffer properties of the pyridine. The presence of 1 ml of water does not affect the indicator noticeably. As much as 5 ml of water can be present as long as an equal amount is added to the blank. Impurities in the hydroxylamine hydrochloride or pyridine may also affect the color of the indicator.

In addition to the reversible color change of the indicator (blue to yellow) with temperature, a gradual permanent shift occurs toward the yellow after prolonged heating at 100°C. This shift is probably due to slow decomposition of the hydroxylamine hydrochloride liberating hydrochloric acid. Heating periods up to 5 hours at 100°C give satisfactory green colors in the blank. No decomposition is noted in the cold even after one month.

Calculations.

$$\frac{\text{Milliliters NaOH} \times N \text{ NaOH} \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 1000} = \% \text{ carbonyl compound}$$

The titration is reproducible to ± 0.1 to 0.2 ml. If optimum-size samples are chosen, the precision of this method is $\pm 1\%$ or better.

Since the method is acidimetric, acidic or basic materials in the sample will interfere. The presence of large amounts of inert organic solvents is undesirable because of their effect on the endpoint. Small amounts of ferric hydroxide or oxide (possibly ferric salts in general) induce a decomposition of reagent and lead to high results.

General References

E. W. Adams and H. Adkins, *J. Am. Chem. Soc.*, **47**, 1358–1367 (1925).
B. G. Feinberg, *J. Am. Chem. Soc.*, **49**, 87–116 (1913).
M. Ripper, *Monatsh.*, **21**, 1079 (1900).

3 Carboxylic Acids, Salts, Esters, Amides

CARBOXYLIC ACIDS

Carboxylic acids are most simply determined by means of a neutralization equivalent. No specific procedure need be quoted here, since the analysis is a simple acid-base titration. However, a few points to be remembered are given to aid in making such a determination.

1. Unknown acids should be titrated, a *p*H meter being used, since the *p*H at the endpoint is not known. Once the *p*H at the endpoint is established for the particular compound a suitable indicator can be chosen.

2. If samples are insoluble in water, they probably will dissolve in alkaline solution. These samples can be dissolved in an excess of standard alkali, and the excess alkali can then be titrated. Water-insoluble samples may also be dissolved in alcohol and titrated with alcoholic sodium hydroxide. Indicators have to be used in these cases since *p*H meters will not operate in alcoholic solutions.

3. Should the acid yield a poor endpoint when titrated, a *p*H meter should be used and the *p*H versus milliliters of alkali curve plotted. The *p*H break may not be sharp enough for an indicator, but the *p*H meter will detect it more readily. If the break is too gradual, the use of stronger standard solutions of alkali will serve to sharpen the endpoint.

4. The procedure of Palit described below for the determination of carboxylic acid salts can be used to determine weak acids by means of sodium hydroxide. The procedure uses special solvent mixtures which accentuate *p*H changes at the endpoint.

SALTS OF CARBOXYLIC ACIDS

There are two methods available for determining carboxylic acid salts. One is direct titration with acid; the other is combustion of the salt to the carbonate and titration of the carbonate with acid.

Adapted from the Method of S. Palit

(*Ind. Eng. Chem.*, Anal. Ed., **18**, 246–251 [1946].)

This method involves the use of special solvent mixtures in which the endpoint during standard acid addition is sharper than in water. These solvents are mixtures of a glycol type solvent and a solvent for hydrocarbons, such as hydrocarbons themselves, alcohols, and chlorinated hydrocarbons. The solutions can be titrated directly with hydrochloric acid or perchloric acid dissolved in the same solvent mixture. Indicators or a *p*H meter can be used to indicate the endpoint.

Glycols are good solvents for salts of carboxylic acids; this solubility is attributed to hydrogen bonding between the solvent and solute. The hydrocarbons in the solvent mixture contribute to the solution of salts of long-chain fatty acids and also contribute to the sharpening of the endpoint. The best solvent mixture for most general applications is ethylene glycol or propylene glycol and isopropyl alcohol. In the determination of salts of long-chain fatty acids, such as sodium stearate, the isopropyl alcohol does not have sufficient dissolving power. More powerful solvents such as butyl or amyl alcohol, chloroform, or dioxane have to be used.

The proportions of the solvents which can be used successfully extend over a wide range; 15 to 70% isopropyl alcohol by volume is satisfactory. However, a 1:1 mixture by volume of ethylene or propylene glycol with isopropanol is used as a standard solvent medium; although, in general, 20% by volume of isopropanol is slightly better as to sensitivity and solubility.

Any free base contained in the carboxylic acid salt can be determined since on addition of the acid the free alkali will yield a break in the *p*H versus milliliters of standard acid curve at a higher *p*H than the salt. A *p*H meter or separate indicators for the free base and the carboxylic acid salt can be used to indicate the two endpoints.

Both hydrochloric and perchloric acids give satisfactory results. The latter is more advantageous in titrating concentrated solutions since the perchlorate formed is more soluble in the special solvent than the chloride and consequently, does not produce a turbidity during the titration.

Procedures.

1. Potentiometric Method. A sample of about 0.004 mole of salt is weighed into a 150-ml beaker together with 40 ml of a 1:1 ethylene glycol–isopropyl alcohol or other appropriate solvent mixture, depending on the material being determined. (See above discussion for details.) The standard Beckman *p*H meter electrodes (glass and calomel) are used. The electrodes are adjusted with an aqueous buffer at *p*H 7. They are then rinsed with water, gently wiped with cleansing tissue, and immediately immersed in the solution. Steady readings are usually obtained in 2 minutes. 0.2 *N* acid (perchloric or hydrochloric) in the same solvent mixture as used for the sample is added from a 25-ml burette. The readings are taken in the usual manner. The *p*H readings have no absolute significance in these solvents, but the sharp break in the *p*H versus milliliters of acid curve is the change sought.

2. Indicator Method. A sample of about 0.004 mole of salt is weighed into a 125-ml Erlenmeyer flask and is dissolved in a minimum of 1:1 ethylene glycol–isopropanol or other convenient solvent. Quick solution can be brought about by adding 10 ml of the glycol to soften the sample, it being warmed if necessary. After the sample is well swollen, an equal volume of isopropanol or other solvent (usually chloroform is used as alternate) is added and the sample is dissolved. Three to five drops of a 0.05% alcoholic solution of methyl red (or methyl orange) are added, and the solution is titrated with standard 0.2 *N* perchloric acid in 1:1 glycol–isopropanol solution to the pink color. (Chloroform sometimes requires a blank because of the free hydrochloric acid it may contain.)

Sodium salts of acetic, propionic, butyric, oleic, stearic, cinnamic, and benzoic acids give satisfactory results by these methods. Salts of acids stronger than formic acid ($Ka = 2.1 \times 10^{-4}$) cannot be titrated without losing some accuracy.

Acidic or basic impurities in the sample will interfere. Weak bases such as aromatic amines can be determined by this method.

Adapted from the Combustion Method of Siggia and Maisch

(*Ind. Eng. Chem.*, Anal. Ed., **20**, 235–236 [1948].)

This method is rather simple in application and principle. The

salt of a carboxylic acid is oxidized to the corresponding carbonate, and the carbonate is determined acidimetrically.

Reagents.

0.5 *N* aqueous sulfuric acid.
0.5 *N* aqueous sodium hydroxide.
Phenolphthalein indicator—methyl red indicator.

Procedure. A sample containing about 0.02 equivalent of carboxylic acid salt is weighed into a platinum crucible. Platinum is used because the carbonate will react with porcelain. The contents are then ignited until all traces of carbon have been burned off. The crucible is cooled and dropped into a 250-ml beaker containing 50 ml of standard 0.5 *N* sulfuric acid. A watch glass is used to cover the beaker and prevent loss of solution by spraying during the evolution of carbon dioxide. The solution is then boiled for 20 to 30 minutes to drive off all the dissolved carbon dioxide. The contents are cooled, and the excess acid is titrated with standard 0.5 *N* sodium hydroxide, phenophthalein indicator being used. In the case of barium and calcium salts, methyl red indicator must be substituted and hydrochloric acid should be used instead of sulfuric acid since it is more efficient in effecting solution of the carbonates. Barium and calcium sulfates, being insoluble, make solution of the carbonate in sulfuric acid difficult. The ignition residues dissolve very readily in hydrochloric acid. However, to avoid loss of hydrochloric acid during boiling, it is best, as soon as the ignition residue is dissolved, to titrate the excess acid with standard alkali. Then the solution is made barely acid with hydrochloric acid. The solution can then be boiled to eliminate the carbon dioxide without appreciable loss of hydrochloric acid. The solution is titrated to the endpoint.

Calculations.

A = milliliters of alkali needed to titrate all standard acid used in analysis

B = milliliters of alkali used in titration of sample

$A - B$ = milliliters of alkali equivalent to carboxylic acid salt = C

$$\frac{C \times N \text{ NaOH} \times \text{mol. wt. salt} \times 100}{\text{Grams sample} \times 1000 \times B} = \% \text{ carboxylic acid salt}$$

B = valence of cation on salt $\times$ number of cation atoms per molecule of salt

To test this procedure these salts were used: sodium acetate, sodium benzoate, sodium citrate, sodium succinate, sodium caprylate, sodium palmitate, sodium laurate, sodium caprate, sodium potassium tartrate, potassium succinate, potassium acid phthalate, calcium acetate, calcium gluconate, calcium citrate, calcium stearate, barium acetate, and barium tartrate.

ESTERS OF CARBOXYLIC ACIDS

Esters may be very simply determined by saponification with standard alkali solutions. Any free acid or base in the sample should be determined beforehand.

Procedure. A sample containing about 0.01 mole of ester is weighed into a 250-ml glass-stoppered Erlenmeyer flask with a condenser to fit, the ground joint for use with volatile or water-insoluble samples. To the sample is added 50 ml of 0.5 *N* sodium hydroxide, aqueous if the sample is soluble in water or alcoholic if the sample is insoluble in water. The solution is heated for 2 hours on a steam bath (longer if the particular ester is difficultly saponifiable). The heating should be under reflux for volatile samples or if alcoholic sodium hydroxide is used. After the specified length of time, the excess alkali is titrated with standard 0.5 *N* acid, phenolphthalein indicator being used.

Calculations.

$$\text{Milliliters of acid to titrate 50 ml of the alkali} - \text{milliliters of acid for sample} = A$$

$$\frac{A \times N \text{ acid} \times \text{mol. wt. ester} \times 100}{\text{Grams sample} \times 1000} = \% \text{ ester}$$

A should be corrected for any free base or acid in the sample.

The procedure was tested with ethyl acetate, diethyl succinate, dibutyl phthalate, glycerol monoacetate, methyl salicylate, methyl acrylate, and ethyl benzoate.

Amides constitute an interference since they will also hydrolyze with alkali to yield carboxylic acid salts. The above procedure can be used to determine many amides. A longer time and stronger alkali for the hydrolysis, however, are generally required.

$$RCONH_2 + NaOH \rightarrow RCOONa + NH_3$$

The liberated ammonia can be dissolved in an excess of standard

acid and the excess acid titrated, or the ammonia can be boiled off and the excess alkali used in the hydrolysis can be titrated.

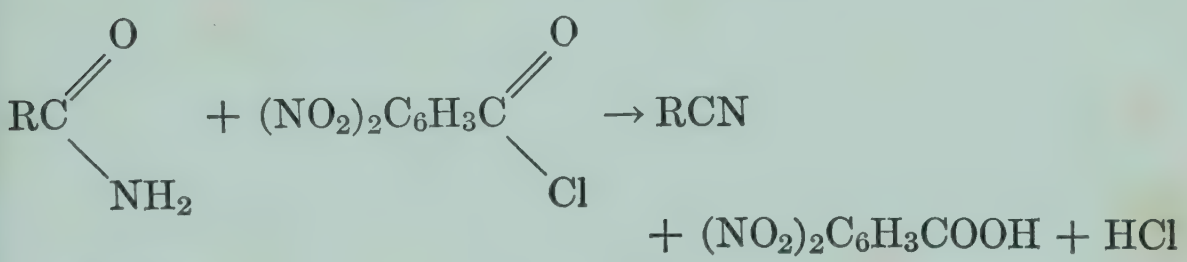
ACID AMIDES (PRIMARY)

These compounds generally cannot be determined by the acetylation or nitrosation methods described for amines (pp. 65–72). Also, they are not basic enough to be titrated with acid as can some amines.

Described below is a procedure for acid amides which involves reaction with 3,5-dinitrobenzoyl chloride in a pyridine solution. Amides can also be determined by hydrolysis with alkali to the sodium salt of the acid and ammonia, as described above.

Adapted from the Method of J. Mitchell, Jr., and C. E. Ashby

(*J. Am. Chem. Soc.*, **67**, 161–164 [1945].)



Reagents.

2 *M* 3,5-dinitrobenzoyl chloride.

The reagent solution is prepared by dissolving 461 g of 3,5-dinitrobenzoyl chloride (Eastman Kodak) in enough purified, anhydrous 1,4-dioxane to yield a liter of solution. The reagent solution is treated with activated carbon and rapidly filtered, care being taken to protect it from exposure to moisture. The final solution should be no darker than light yellow or else there will be difficulty in determining the endpoint color change.

C.P. dry pyridine.

C.P. dry methanol.

0.5 *N* sodium methoxide in methanol prepared directly from sodium in anhydrous methanol. This solution should be standardized each day by titrating an aliquot with 0.5 *N* standard acid, phenolphthalein indicator being used, or ethyl bis-2,4-dinitrophenyl acetate indicator (a saturated solution in 1:1 acetone-ethyl alcohol).

Procedure. A sample containing about 10 milliequivalents of amide is weighed into a 250-ml glass-stoppered Erlenmeyer flask containing 15 ml of the 3,5-dinitrobenzoyl chloride reagent and 5 ml of pyridine. The flask together with a blank is placed in a water bath at 60°C for 30 minutes (70°C for one hour when amides

of dibasic acids are determined). At the end of this time the flasks are removed and cooled in ice. The excess aroyl chloride is decomposed with dry methanol, which is added in two portions, first 2 ml and, after 5 minutes, an additional 25 ml. The solution and blank are then titrated with 0.5 *N* standard sodium methoxide, ethyl bis-2,4-dinitrophenyl acetate indicator being used. Phenolphthalein can be used, but the endpoint is not so sharp as with the dinitrophenyl acetate because of the yellow-orange color of the solution.

The net increase in acidity of the sample over the blank, after correction for free acid and water present in the original sample, is equivalent to the quantity of primary amide present.

Calculations.

$$\text{Milliliters sample} - \text{milliliters blank} = A$$

$$\frac{A \times N\ CH_3ONa \times \text{mol. wt. amide} \times 100}{\text{Grams sample} \times 1000} = \%\ \text{primary acid amide}$$

Interfering substances in the above procedure consist mainly of free acid and water. These materials should be determined in the original samples and the corrections applied to the results of the amide analysis. The water hydrolyzes the acid chloride to liberate 3,5-dinitrobenzoic acid and hydrochloric acid. Secondary and tertiary amides generally do not interfere in the analysis. Amines and alcohols do not affect the results beyond the fact that they may inactivate some of the reagent.

To test this procedure the following primary amides were used: formamide, acetamide, propionamide, butyramide, isobutyramide, *n*-valeramide, heptamide, succinamide, glutaramide, adipamide, benzamide, salicylamide, *p*-nitrobenzamide, phthalamide, furoamide.

The procedure is generally accurate and precise to ± 0.5 to 1.0%.

4 Alkoxyl and Alkimide Groups

The alkoxyl and alkimide groups are restricted to the methyl and ethyl members of these groups. The higher homologs are difficult to determine, but by modifying the alkoxyl apparatus (see Figs. 2 and 3) propoxyl and butoxyl groups can be determined.

Both methods involve the use of hydriodic acid. In the alkoxyl determination, the alkyl group is hydrolyzed, forming the corresponding alkyl iodide (see equation below). The alkyl iodide is collected and determined.

The alkimides are determined by a pyrolysis reaction of the alkimide with hydriodic acid. The resulting alkyl iodide is determined as in the alkoxyl groups.

ALKOXYL GROUPS—METHOXYL AND ETHOXYL

Combined Methods of Elek. Ind. Eng. Chem., Anal Ed., 11, 174 (1939) and Samsel and McHard, Ind. Eng. Chem., Anal. Ed., 14, 754 (1942).

(Adapted with permission from the description in Niederl and Niederl's *Micromethods of Quantitative Organic Analysis*, 2d Ed., pp. 239–244, John Wiley and Sons, New York, 1942.)

(*a*) $\left.\begin{matrix} ROR' \\ RC(=O)OR \end{matrix}\right\} \xrightarrow{HI} RI$

(*b*) $RI + Br_2 \rightarrow RBr + IBr$

(*c*) $IBr + 3H_2O + 2Br_2 \rightarrow HIO_3 + 5HBr$

(*d*) $HIO_3 + 5HI \rightarrow 3I_2 + 3H_2O$

Reagents.

Potassium acetate in acetic acid. Dissolve 100 g of c.p. anhydrous potassium acetate in 1 liter of solution containing 900 ml of glacial acetic acid and 100 ml of acetic anhydride.

Bromine solution. Dissolve 1 ml of bromine in 29 ml of the above potassium acetate reagent. This solution should be prepared fresh daily.

Sodium acetate. Dissolve 250 g of c.p. anhydrous sodium acetate in 1 liter of distilled water.

Aqueous red phosphorus suspension. Suspend 30 g of c.p. red phosphorus in 50 ml of water.

Potassium iodide, c.p.

Formic acid, reagent grade, 90% sp. gr. 1.20.

Hydriodic acid. C.p. constant boiling mixture, b.p. 126 to 127°C (57% hydriodic acid).

10% sulfuric acid. Add 60 ml of conc. sulfuric acid to 940 ml of distilled water.

0.1 *N* standard sodium thiosulfate.

Starch indicator.

Carbon dioxide. A commercial cylinder with a reducing valve is a good source.

Apparatus (Fig. 2).

Complete apparatus can be purchased from Scientific Glass Apparatus Co., Bloomfield, New Jersey, Catalog M-1745.

Procedure. The trap (*A*) is two-thirds filled with the red phosphorus suspension. The phosphorus trap is to keep any free iodine from getting into the receiver.

$$2P + 3I_2 \rightarrow 2PI_3$$
$$PI_3 + 3H_2O \rightarrow 3HI + H_3PO_3^*$$

The receiver (*B*) is filled to a level representing one-third of the bulb portion (*C*) with bromine solution. The sample is placed in the reaction vessel together with a boiling chip and a crystal of phenol. Liquid samples should be weighed in gelatin capsules. To the sample is added 6 ml of constant boiling hydriodic acid, the reaction flask is immediately attached to the condenser, and the joint is moistened with hydriodic acid. Water is circulated through the condenser, and the reaction flask is heated. A slow stream of carbon dioxide is bubbled through the solution at a rate of about 2 bubbles per second. An oil bath at 130 to 150°C or a hot-spotter can be used as the heating element. After 45 minutes (one hour for ethoxyl determination) the contents of the receiver are quantitatively transferred to a 500-ml flask containing 10 ml of sodium acetate solution. The solution is diluted to about 125 ml with water, and formic acid is added dropwise until the bromine color is discharged; then 3 additional drops are added. After the

* Samsel and McHard, *Ind. Eng. Chem.*, Anal. Ed., **14**, 754 (1942).

solution stands 3 minutes, 3 g of potassium iodide and 15 ml of 10% sulfuric acid are added. The liberated iodine is titrated with 0.1 *N* sodium thiosulfate, starch indicator being used. A blank should be run on the phenol alone. In most cases, however, the blank is so small as to be unnecessary.

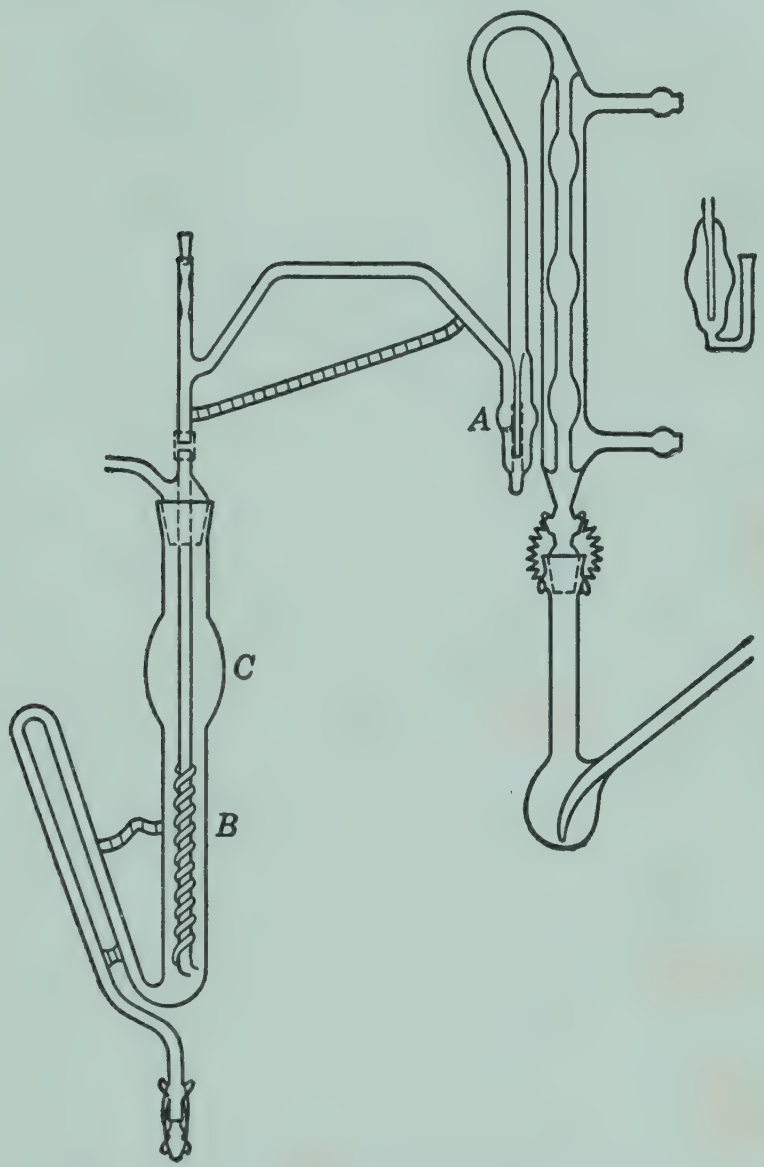


FIGURE 2. Methoxyl-ethoxyl apparatus.

In a few cases the sample is not attacked completely by the hydriodic acid. This is probably due to insolubility in the acid. In these cases, a 1:1 phenol–hydriodic acid mixture should be tried.

Calculations.

$$\frac{\text{Milliliters thiosulfate} \times N \text{ thiosulfate} \times \text{mol. wt. alkoxyl group} \times 100}{\text{Grams sample} \times 6000} = \% \text{ alkoxyl}$$

The above procedure is reproducible and accurate to ±0.2%. Methyl and ethyl alkimides interfere since they are determined in a similar manner. Propoxyl and butoxyl groups also interfere because their iodides, which are formed, are sufficiently volatile to be partially distilled with the methyl or ethyl iodide, causing high results.

The procedure was tested with ethoxy- and methoxybenzene; dimethylformal; dimethylacetal; 1,1,3-trimethoxybutane; sodium ethylate; 2,4-dimethoxy-2-methylpentane.

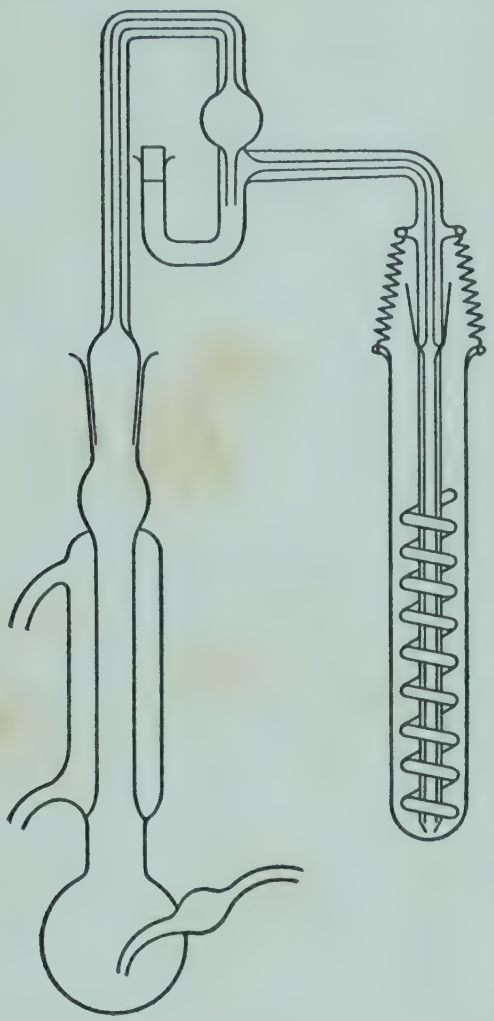


FIGURE 3. Propoxyl-butoxyl apparatus.

PROPOXYL-BUTOXYL GROUPS

The method described for the determination of methoxyl and ethoxyl groups can be applied in this case if the apparatus is modified as shown in Fig. 3. This modification (Shaw, *J. Soc. Chem. Ind.*, **66**, 147–149 [1947]) makes it possible to distil the propyl and butyl iodides quantitatively into the receiver by eliminating a great deal of the tubing connecting the reactor and the receiver.

ALKIMIDE GROUPS

Methyl or ethyl groups on the nitrogen atom of amines can be split off as the alkyl iodide with hydriodic acid. The hydriodic acid first reacts to form the alkyl ammonium iodide, then this salt is split thermally to liberate the alkyl iodide, which is distilled and trapped as in the above alkoxyl analysis.

$$\rangle N{-}R + HI \rightarrow \rangle N{-}R{\cdot}HI \xrightarrow{\text{Heat}} RI$$

(Remaining equations are the same as those in alkoxyl section, p. 27.)

Method Described by Niederl and Niederl

(Adapted with permission from *Micromethods of Quantitative Organic Analysis*, 2d Ed., pp. 244–246, John Wiley and Sons, New York, 1942.)

Reagents.

In addition to the reagents listed in the alkoxyl procedure, the following are necessary:

5% aqueous gold chloride.
Ammonium iodide, c.p.
Propionic acid anhydride.

Apparatus (Fig. 4).

Can be purchased from Scientific Glass Apparatus, Bloomfield, N. J. (except that spiral bubbler is lacking). M 2675, the receiver containing spiral bubbler from methoxyl apparatus, can be purchased and attached.

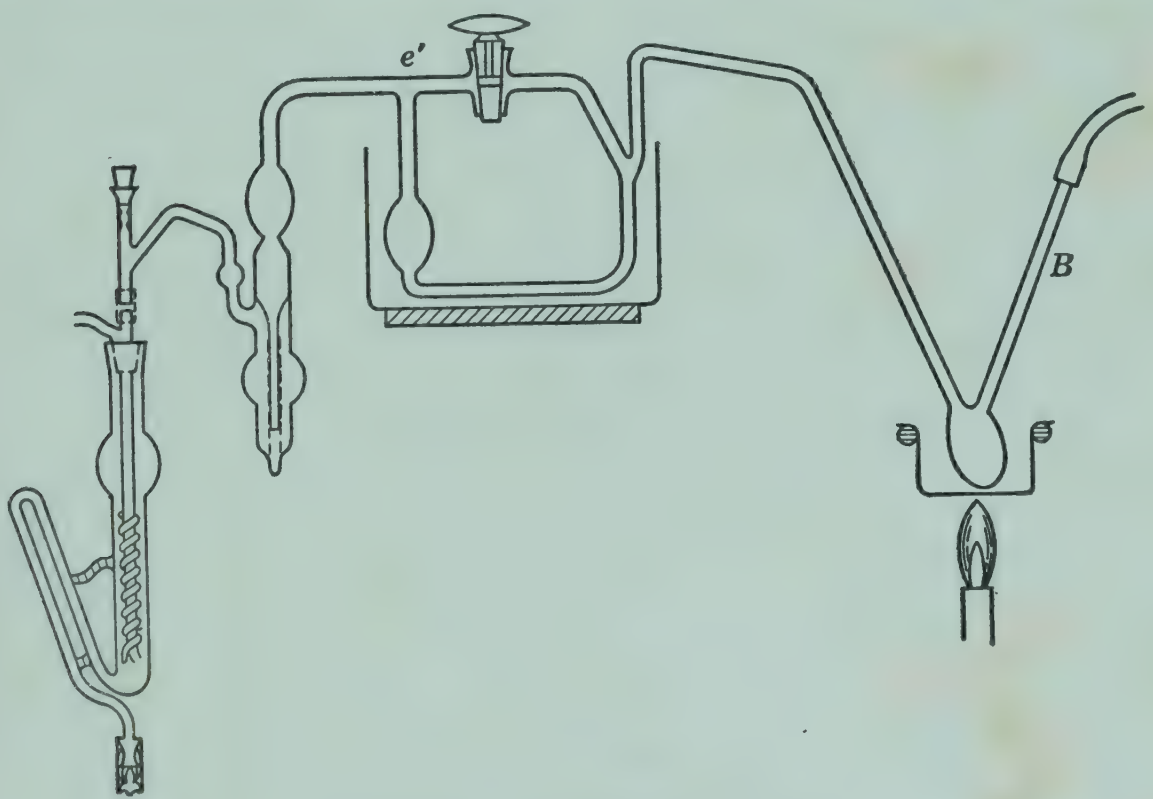


FIGURE 4: Alkimide apparatus.

Procedure. The trap in this apparatus, as in the alkoxyl determination, contains the phosphorus suspension, and the receiver contains the same bromine solution. The sample is added to the reaction flask. A few crystals of phenol are added, and propionic acid anhydride is added dropwise (6 to 10 drops) until the sample is dissolved. If the sample does not dissolve, it should be heated. If solution is still not achieved, additional phenol and propionic

anhydride are added until the sample does dissolve (a corresponding increased amount of hydriodic acid should be added in these cases). A spatula pointful of ammonium iodide is added, followed by 1 or 2 drops of gold chloride solution, followed by 3 ml of hydriodic acid. The reaction flask is then attached to the condenser, and the joint is moistened with hydriodic acid. The rate of the carbon dioxide stream should be about 2 bubbles per second.

The rectangular portion of the apparatus (see diagram) is immersed in a water bath at 90°C. The stopcock is in position as indicated in the diagram. The reaction vessel is placed in a metal pan containing copper oxide in which a thermometer is immersed. The pan is heated gradually to 360°C; this gradual heating should require about one hour. The heating at 360°C should be continued until all the hydriodic acid is distilled. The heat is turned off, and the apparatus is allowed to cool to room temperature. The stopcock is then turned clockwise until the vertical groove is connected to arm e'. The flow of carbon dioxide is turned off and the rubber tube on B is removed, and the hydriodic acid in the lower part of the rectangle is sucked back into the reaction flask. About 0.5 ml of fresh hydriodic acid is added by means of the vertical opening of the stopcock, and this is also sucked into the reaction flask. The receiver is exchanged for a freshly filled one, the stopcock is turned to the original position, and the carbon dioxide is again turned on. The heating is repeated at least once more as described above.

The remainder of the analysis is carried out as described in the alkoxyl section.

5 Unsaturation (—C=C— ; —C≡C—)

There are two common methods of determining carbon-carbon unsaturation. One is bromination, the other is catalytic hydrogenation.

The bromination procedures for determining phenols and amines (see p. 111) cannot usually be used to determine unsaturation since the reactions are too slow and not quantitative. A procedure has been devised with mercuric sulfate as a catalyst for the addition. Some compounds will brominate without the catalyst, but they are very few. Some compounds, such as cinnamic acid, are best determined without the catalyst since its presence causes much substitution to take place together with the addition.

Samples containing unsaturated linkages can be hydrogenated by using platinum oxide catalyst (Adam's catalyst) or Raney nickel. With ordinary precautions the method is reliable to ±5%. By making special temperature corrections, results can be obtained which are reproducible to ±2%. This method is ideal for determining the number of double bonds per molecule but, because of its limited reproducibility, it is not ideal for assaying samples. However, if the unsaturated compound has no other functional group which can be used to assay it, this hydrogenation method can be used if the reproducibility of ±2% is kept in mind.

The hydrogenation method is more general for unsaturation than bromination. There are no substitution difficulties when adding hydrogen as there are when adding bromine. However, hydrogenations take longer to carry out than brominations, and greater precautions are necessary for good results, as well as for safety.

Platinum oxide catalyst generally causes hydrogenations to proceed more rapidly than Raney nickel. Under the conditions employed, platinum oxide will saturate the double bonds in a benzene ring whereas Raney nickel will not. This fact should be kept in mind in calculating results.

BROMINATION

Adapted from the Method of H. J. Lucas and D. Pressman

(*Ind. Eng. Chem.*, Anal. Ed., **10,** 140–142 [1938].)

Reagents.

0.1 *N* bromate-bromide solution.
0.2 *N* mercuric sulfate.
C.p. carbon tetrachloride.
C.p. glacial acetic acid.
20% potassium iodide.
6 *N* sulfuric acid.
0.05 *N* sodium thiosulfate.
Starch indicator solution.
2 *N* sodium chloride.

Procedure. Water-soluble samples are diluted to 0.08 *N* in unsaturation and a 25-ml aliquot taken (0.002 equivalent in unsaturation).

Hydrocarbon-soluble samples are dissolved in carbon tetrachloride.

When volatile, the sample is weighed in a sealed glass ampoule (see Section 25) and placed in a volumetric flask of convenient volume to give a 0.08 *N* solution in terms of unsaturation, along with enough carbon tetrachloride or water to cover the ampoule. The volumetric flask is chilled in ice water, and the ampoule is then broken with a glass rod. The rod is rinsed with chilled carbon tetrachloride or water, and the solution is made up to the mark with the same chilled solvent. To pipette a sample of this solution, vacuum should not be applied. Instead, the pipette is equipped with a 2-hole rubber stopper to fit the volumetric flask, and a piece of glass tubing is inserted in the other hole. The solution is then blown into the pipette by compressed air or by mouth.

A calculated excess (10 to 15%) of 0.1 *N* bromate-bromide solution (about 25 ml) is introduced into a 250 to 300-ml Erlenmeyer flask having a glass stopper equipped with a sealed-in three-way stopcock (see Fig. 5). If the amount of unsaturation in the sample is unknown, a preliminary analysis is made with a large excess of bromate-bromide solution. From this result the desired excess can be calculated. The reason for avoiding the large excess is to minimize substitution, which leads to high results. If the excess is less than 10 to 15% the addition proceeds so slowly toward the end of the bromination that the bromination may not be complete in the specified time.

After the addition of the bromate-bromide solution the flask is evacuated through tube *A* with a water aspirator, and 5 ml of 6 *N* sulfuric acid is added by means of the funnel attachment *B* on

the stopper, 2 or 3 minutes being allowed to elapse for the bromine to be liberated. Next, 10 to 20 ml of 0.2 *N* mercuric sulfate is added, followed by the sample containing solution (25 ml of the sample solution should be used if the concentration is 0.08 *N* with respect to unsaturation). Water or carbon tetrachloride, depending on which was used to dissolve the sample, should be used to rinse the sample into the flask. A total of 15 ml of wash liquid in three portions should be employed. In the case of samples dissolved in carbon tetrachloride, 20 ml of glacial acetic acid is added. When the samples are dissolved in water the acetic acid is omitted. The flask is then wrapped in black cloth and is shaken for 7 minutes (time may vary with some samples). Then 15 ml of 2 *N* sodium chloride and 15 ml of 20% potassium iodide are added and the flask shaken for about one-half minute. The vacuum is broken, and the free iodine is titrated with 0.05 *N* sodium thiosulfate, starch indicator being used. A blank, with about one-third the amount of bromate-bromide solution used in the analysis, is run under the same conditions as the samples.

FIGURE 5. Bromination apparatus.

Calculations.

$$\frac{(\text{Milliliters for blank} - \text{milliliters for sample}) \times N \text{ thiosulfate} \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 2000 \times B} = \% \text{ compound}$$

B = number of moles bromine absorbed by compound being determined

The molar ratio of mercuric ion to bromide ion should be greater than one if the mercuric salt is to have sufficient catalytic effect. Sodium chloride is necessary to liberate free bromine from its

complex with mercuric sulfate. Acetic acid is necessary to solubilize the unsaturated compound in the water layer.

The 7-minute shaking is enough for most samples, but some samples require a longer time (Table 3). Maleic and fumaric acids in water require one-half hour in the presence of mercuric sulfate for complete reaction.

Table 3

Effect of Variations in the Analytical Procedure on Results

Compound	$HgSO_4/Br^-$, ratio	HOAc, ml	$KBrO_3$ Excess, %	Time, minutes	Error, %
		Mercury-Bromide Ratio			
2-Heptyne	2.4	20	20	7	−3.2
2-Heptyne	1.2	20	20	7	−4.1
2-Heptyne	0.0	20	20	7	−23.0
		Acetic Acid			
Phenylacetylene	1.1	25	10	7	−3.0
Phenylacetylene	1.1	0	10	7	−23.0
1-Hexyne	1.1	25	10	5	−0.9
1-Hexyne	1.1	0	20	5	−10.0
		Excess Bromate			
1-Pentyne	1.0	0	25	20	+12.0
1-Pentyne	1.7	0	12	20	+7.5
1-Pentyne	1.7	0	5	20	+5.5
		Time of Bromination			
1-Heptyne	1.5	15	15	60	−2.1
1-Heptyne	1.5	15	15	30	+2.7
1-Heptyne	1.5	15	15	15	+2.7
Phenylacetylene	1.1	20	10	30	+3.7
Phenylacetylene	1.1	20	10	5	0.0

Cinnamic acid requires no mercuric sulfate for quantitative results; in fact, mercuric sulfate is detrimental since it causes substitution. Propiolic acid, dimethylbutadiene, and propargyl alcohol substitute bromine and yield high results when determined by this method. Because of varying amounts of substitution for different compounds, it is difficult to ascribe a general accuracy and precision value to this procedure.

Absorbed oxygen in solutions of alkynes and exposure to sunlight generally affect results significantly.

This procedure was tested with phenylacetylene, 1-heptyne, 1-pentyne, 1-hexyne, cyclohexene, 1-hexene, dichloroethylene, maleic acid, fumaric acid, 2-butyne-1,4-diol, anethole.

Interfering substances consist of phenols and amines, which substitute bromine, and also hydrazines, some aldehydes, and other materials which are oxidized by bromine.

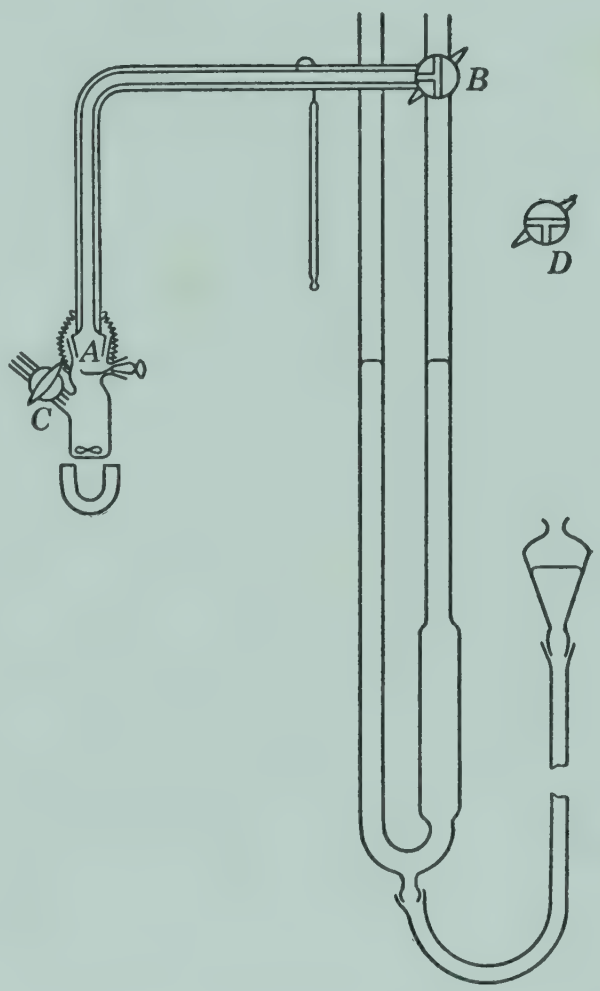


FIGURE 6. Quantitative hydrogenation apparatus.

HYDROGENATION

Procedure. About 3 ml of solvent and about 0.5 g of catalyst are added to the hydrogenation vessel. The amount of catalyst is not critical. If a larger amount of catalyst is used, the hydrogenation of the compound proceeds more rapidly. The time required to saturate the catalyst, however, is much longer, so that no time is saved. When a small amount of catalyst is used, the sample hydrogenates more slowly, but the time is saved in saturating the catalyst.

A weighed sample which will consume about 0.0002 mole of hydrogen is put in the cup indicated by *A* on the diagram (Fig. 6). If the sample is a solid it is weighed in a small aluminum boat or envelope. The boat is placed in the cup. Liquid samples are weighed in small glass receptacles or in gelatin capsules. (When using capsules, water must be used as the solvent.) All joints and stopcocks should be well greased to prevent hydrogen leakage.

The system is flushed with a slow stream of hydrogen for 3 to 5 minutes, introducing the hydrogen through stopcock at *B*, which is in position as indicated; the mercury level is as near the stopcock as possible. The hydrogen is allowed to escape through

stopcock *C*. After 3 to 5 minutes stopcock *C* is closed and the mercury is lowered to a position well below the calibrations on the burette. Then stopcock *B* is turned to position *D* and the hydrogen is disconnected.

A pressure of about 3 cm of mercury is applied and the stirrer started. Stirring is brought about by a rotating magnet under the reaction flask with a small glass-covered iron paddle inside the flask. These magnetic stirrers can be purchased or they can be improvised by attaching a magnet to the shaft of a stirring motor. The more vigorous the agitation, the more rapidly will the catalyst be saturated and the sample hydrogenated. When the mercury level ceases to rise, it signifies that the catalyst is saturated. The leveling bulb is then raised and stopcock *C* is opened slowly, allowing hydrogen to escape until the mercury level is within the calibrations on the gas burette. The stopcock is again closed and about 5 to 10 minutes are allowed for the apparatus to come to equilibrium. The burette reading and the temperature are noted. The sample is then allowed to fall into the solvent by turning *A*. About 3 cm pressure is applied as agitation is continued, until the level of the mercury ceases to rise. The burette reading is taken and 3 cm pressure again applied for 10 minutes to insure complete reaction. The temperature is again noted.

For accurate results, the volume of the apparatus is needed to correct hydrogen volume readings when the temperature at the end of an analysis is different from the temperature at the beginning. If this is not done the volume readings will be in error because of expansion or contraction of the hydrogen in the free space of the apparatus. The volume of the apparatus is measured by completely filling it (up to the 7-ml mark on the burette) with water and then weighing the water. The correction is made by adding the volume of the apparatus to the volume between the 7-ml mark and the level of the mercury at the end of the hydrogenation minus the volume of solvent used. This equals the volume of free space at the end of the hydrogenation. This volume is corrected for temperature changes which may have occurred during the determination by the following equation:

$$\frac{V_1}{T_1} = \frac{V_2}{T_2} \qquad (T = \text{absolute temperature})$$

The difference between the volume before the temperature correction and the volume after the correction is the volume change due to expansion or contraction of the gas in the apparatus. This volume change is either added or subtracted from the volume of hydrogen consumed as read on the burettes, depending on whether the final temperature was lower or higher than the initial temperature.

Low-boiling solvents are to be avoided since temperature changes affect the vapor pressure of the solvent. Since the low-boiling solvents exhibit significant variations in vapor pressure over the range of temperatures encountered in the room, significant errors can result in the volume measurements.

The procedure as described above will yield results with an accuracy and precision of $\pm 5\%$. If a 50-ml gas burette is used and the sample is five to eight times larger (the remainder of the apparatus remaining exactly the same), the results will be accurate and precise to ± 1 to 2%.

Oxygen should be completely eliminated from the apparatus since it will consume hydrogen. Any other materials which can be reduced with hydrogen will of course interfere.

Calculations.

Volume of hydrogen consumed (corrected for any temperature fluctuations) $= V_t$
Volume of hydrogen converted to 0°C $= V_0$
The temperature of the experiment $= T$°C $+ 273$
The temperature at 0°C $= 273$

$$\frac{V_t}{T\text{°C} + 273} = \frac{V_0}{273}$$

$$\frac{V_0/22{,}400}{\text{Grams sample/mol. wt. sample}} = \frac{\text{moles hydrogen consumed}}{\text{mole sample}}$$

Materials used to test the procedure were cinnamic acid, maleic acid, *n*-butyl, and *n*-propyl vinyl ethers, 2-butyne-1,4-diol, propargyl alcohol, and methyl acrylate.

CAUTION

All Raney nickel should be stored under alcohol (preferably a high-boiling alcohol). Dry Raney nickel will react with atmos-

pheric oxygen, emitting sparks which can ignite any surrounding combustible material. The presence of hydrogen increases the hazards. None of the catalyst should be allowed to dry in the apparatus, on the desk top, or on the implements used to introduce the catalyst into the apparatus.

Adam's catalyst when dry will glow in the presence of hydrogen and oxygen. Hydrogen is adsorbed on the catalyst and reacts with oxygen, emitting much heat. When the catalyst is put in the apparatus, it should be completely wet with solvent. Any dry particles of catalyst will glow as soon as the hydrogen is introduced, causing either the solvent vapors to flash or the hydrogen-air mixture to explode.

6 Active Hydrogen Compounds and Compounds Which React with Grignard Reagent

For our purposes we may define an active hydrogen atom as a hydrogen atom which is attached to any other atom except a carbon atom. Compounds which contain active hydrogen atoms and can be determined by reaction with Grignard reagents are alcohols, amines, amides, phenols, mercaptans, acids, sulfonamides, and water.

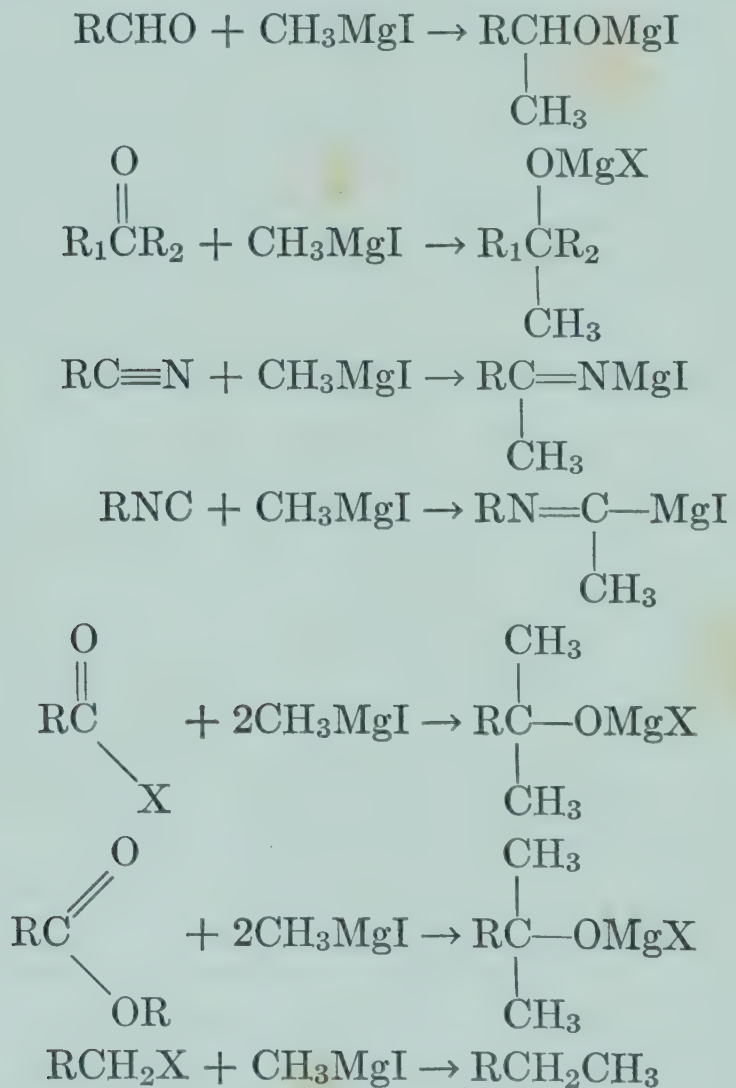
The method consists of reacting the compounds containing active hydrogen atoms with methylmagnesium iodide, and collecting and measuring the methane liberated.

ROH		CH_4
RNH_2		$2CH_4$
R_1 \ NH / R_2		CH_4
O ‖ $RC—NH_2$		$2CH_4$
O ‖ $RC—NHR$		CH_4
ϕOH	$+ CH_3MgI \rightarrow$	CH_4
RSH		CH_4
$RCOOH$		CH_4
RSO_3H		CH_4
RSO_2NH_2		$2CH_4$
RSO_2NHR_1		CH_4
H_2O		$2CH_4$

Although the procedure is reproducible to only about $\pm 3\%$ and can be considered only semi-quantitative, it is useful in determining the number of active hydrogen atoms on a molecule. However,

it can be used for materials where no other analytical methods are available as long as the limitations are kept in mind.

This same method can also be used to determine compounds which add to or couple with the Grignard reagent. A known amount of methyl Grignard reagent is added to the sample and allowed to react. Then the excess Grignard reagent is decomposed and the methane collected and measured. The amount of reagent consumed is a measure of the compound being determined. Compounds which add to or couple with Grignard reagent are aldehydes, ketones, nitriles, isonitriles, acid halides, esters, and alkyl halides.



Method Described by Niederl and Niederl

(Adapted with permission from *Micromethods of Quantitative Organic Analysis*, 2nd Ed., pp. 263–272, John Wiley and Sons, New York, 1942. The apparatus has been modified to simplify handling and increase the accuracy of the method.)

Reagents.

0.4 to 0.5 *M* methylmagnesium iodide:

In a 250-ml round-bottomed distilling flask, equipped with an inlet tube (sealed in about an inch from the neck) and a ground-glass joint with reflux condenser to fit, is placed about 0.6 g of magnesium turnings. To the magnesium turnings are added 2.5 ml of methyl iodide and 50 ml of freshly distilled *n*-amyl ether (the ether should be distilled from and stored over metallic sodium). Nitrogen is passed through the system at a rate of about one bubble per second by means of the inlet tube on the reaction flask. The nitrogen should be of 99.9% purity (Siefert nitrogen). The Grignard reaction is started by dropping a crystal of iodine into the reaction mixture and warming on a water bath. Should the Grignard reaction still fail to start, 2 to 3 ml of absolute diethyl ether is put in a small test tube with a few magnesium turnings, and to this is added 2 to 3 drops of methyl iodide. The reaction starts rapidly in the diethyl ether. As soon as the reaction in the test tube is proceeding at a vigorous rate, the contents of the test tube, while still reacting, are added to the reaction mixture in the flask. This procedure will usually induce the reaction to begin. If, even after this priming, the reaction does not begin, this method can be employed. The reaction mixture is prepared as above except that 25 ml of absolute diethyl ether is used instead of the amyl ether. This reaction begins immediately and is allowed to proceed to completion. Then 50 ml of amyl ether is added by means of the condenser, and the ethyl ether is boiled off as described below. However, this method of preparing the Grignard reagent is seldom needed. The priming of the reaction with the iodine crystal or with some methylmagnesium iodide in ethyl ether is usually enough to get the reaction started.

Once the reaction is started in amyl ether, it is allowed to proceed for two hours while heating gently on a steam bath or until the reaction has appreciably subsided. The steam is then turned off, and the flow of water in the condenser and the flow of nitrogen are stopped. The condenser outlet is connected by means of a rubber tube to a vacuum pump or an efficient aspirator (both equipped with dry ice traps to trap the vapors coming over). A pinch clamp is attached to the rubber tube leading to the source of vacuum in order to regulate the vacuum. The vacuum is turned on, and the pinch clamp is slowly opened, causing the contents of the flask to boil. As soon as the pinch clamp is fully opened, the nitrogen flow is resumed at a rate of about one bubble per second for about 15 minutes. This procedure removes any unreacted methyl iodide and any ethyl ether which may have been added to initiate the reaction. Little amyl ether is lost during the evaporation.

After the evaporation, the rubber tube from the condenser to the source of vacuum is carefully removed while the apparatus is allowed to fill up with nitrogen. The methylmagnesium iodide is then decanted quickly through a funnel containing a glass wool plug into a long-stemmed glass ampoule of the type used for liquid bromine, acetaldehyde, acetyl chloride, etc. The top of the ampoule is sealed with a soft rubber stopper. The

ideal stopper is a serum cap such as is used on ampoules of serum and certain medicinals. The stopper has to be of such a nature that a hypodermic needle can easily be inserted through it. The reagent should be about 0.4 to 0.5 molar. The strength of the reagent can be ascertained by adding excess 0.1 *N* hydrochloric acid to a 5-ml sample of reagent. The excess of acid is titrated with 0.1 *N* sodium hydroxide, using phenolphthalein as an indicator.

Solvents.

Anethole, pyridine, and xylene have been found to be convenient solvents. They should be redistilled and should be thoroughly dry.

Apparatus (Fig.7).

A reaction vessel of about 10-ml capacity.
B 3-way stopcock.
C rubber serum cap.
D 2-mm capillary.
E 7-ml gas burette.
F 3-way stopcock.
G thermometer.

Procedure. A sample containing about 0.0001 to 0.00015 equivalent of active hydrogen is weighed into the reaction vessel *A* and dissolved in 1.0 ml of one of the solvents mentioned. The solvent should be measured with a pipette. A glass-encased iron paddle is dropped into the solution. All the apparatus should be thoroughly dry. After each determination it is best to rinse the reaction flask with acetone and then with ethyl ether; the stopcock should be taken out of the apparatus and all grease removed. All the parts should then be dried in air until all the ether has evaporated; then the parts should be put in an oven at 130°C for at least 15 minutes before the next experiment.

After the sample and solvent are added to the reaction flask, the top edge of the ground-glass joint is greased and the reaction flask is joined to the rest of the apparatus; the top edge of the ground joint must be completely sealed by the grease. The stopcock on the reaction flask should be lubricated with a minimum of grease. There should be no grease in the bore of the stopcock. Nitrogen which has been bubbled through concentrated sulfuric acid is passed through the system for 5 minutes. The nitrogen is introduced through stopcock *F*, which is in position 1*F* and the mercury in the gas burette is kept as close to the stopcock as possible. The nitrogen escapes through stopcock *B*, which is in

position 1*B*. While the nitrogen is still flowing, the mercury level is slowly lowered to the beginning of the calibrations and the serum cap is put over the end of the exit tube on the reaction flask at *C*. Stopcock *B* is then turned to position 2*B*; stopcock *F* is turned to position 2*F*, and the flow of nitrogen is discontinued.

A hypodermic syringe of about 2-ml capacity with a wide bore needle is used for introduction of the Grignard reagent. The

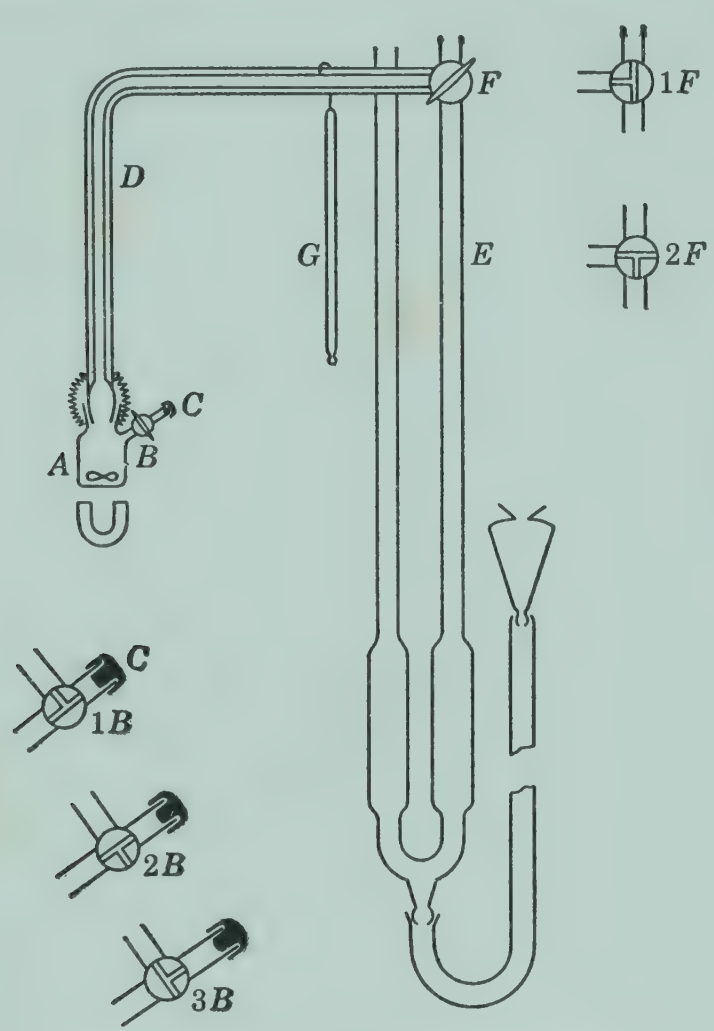


FIGURE 7. Active hydrogen apparatus.

needle is inserted through the serum cap on the ampoule containing the Grignard reagent, and the ampoule is tilted until the reagent solution comes in contact with the needle. The hypodermic is filled with reagent; it is then inverted and the air is driven out until reagent comes through the needle tip. With the mercury levels equalized and the initial reading recorded, the needle is quickly inserted through the serum cap at *C* on the reaction flask and the needle is passed through the bore of the stopcock into the body of the flask. Reagent to the amount of 0.5 to

1.0 ml (or an amount in excess of the theoretical amount required) is injected into the solution. The temperature of the apparatus and the volume of reagent added should be carefully noted and recorded. The needle is carefully withdrawn, and as soon as the tip is past stopcock *B*, the stopcock is turned to position 3*B*. The mercury level is lowered, and the reaction flask is immersed in boiling water for 15 minutes. Then the water bath is removed, and the magnetic stirrer is attached. The reaction mixture is stirred until the mercury level becomes constant. The temperature is again noted along with the mercury level reading.

A blank determination is made on the solvent used for the analysis to determine how much methane is liberated by the solvent. The blank is analyzed by the same method as the sample. It is advisable to use about 5 ml of solvent. The result is divided by 5 to get the blank per milliliter of solvent. If the number of groups which *add* Grignard reagent is to be determined, the strength of the reagent has to be determined. It can be done together with the blank. One ml of solvent is used, a known amount of reagent is added, and the blank is determined. For accurate work, the blank should not be greater than 0.4 ml of methane per milliliter of solvent. Then a measured amount of aniline (about one ml) is injected into the reaction flask in the same manner as the reagent (each time an injection of fluid is made into the reaction flask, the mercury levels should be equalized to prevent leaks), and the contents are stirred until the mercury level becomes constant. The methane liberated by the solvent plus the methane liberated by the aniline corresponds to the strength of the Grignard reagent.

In analyzing a sample which adds Grignard reagent, the reagent is added to the sample and the active hydrogen is determined as described above. After the mercury level is read, the excess Grignard reagent is decomposed with aniline as described. After the level of the mercury has become constant, the level is read along with the temperature.

All parts of the apparatus can be cleaned of precipitated magnesium salts by using dilute hydrochloric acid.

For accurate work, the volume of the entire apparatus is measured by filling all parts of the apparatus with water and weighing the water (see Hydrogenation, pp. 38–39). This is to correct for any temperature changes which occur between burette

readings. Any temperature changes cause errors in volumes because of expansion or contraction of the gas in the free space in the apparatus. After each burette reading, the temperature is noted. If the temperature is different from the initial burette reading, the readings are corrected to the temperature of the initial burette reading before the calculations are carried out. (See section on hydrogenation, p. 38, for the method of correcting for temperature changes.)

Calculations.

A = volume of Grignard reagent added.

B = blank on the solvent.

C = volume of aniline added.

D = initial burette reading.

E = burette reading after reaction with the Grignard reagent (corrected for temperature as indicated above).

F = burette reading after reaction of aniline with excess Grignard reagent (corrected for any temperature change).

G = total methane which would be evolved by the amount of Grignard reagent used, as calculated from the strength of the Grignard reagent predetermined as described above.

$E - D$ = volume change recorded = H

$H - B - A$ = volume of methane liberated by active hydrogen = I

$F - E - C$ = volume of methane evolved by the excess Grignard reagent (reaction with aniline) = J

$G - J$ = volume of methane corresponding to reagent added onto the sample = K

$$\frac{I \times 273 \times \text{mol. wt. compound}}{(T + 273) \times 22{,}400 \times \text{grams sample}} = \frac{\text{atoms of active hydrogen}}{\text{mole sample}}$$

T = temperature of the experiment

$$\frac{K \times 273 \times \text{mol. wt. compound}}{(T + 273) \times 22{,}400 \times \text{grams sample}} = \frac{\text{moles of Grignard reagent added to the sample}}{\text{mole sample}}$$

The above procedure requires some practice to operate successfully. There are several sources of error. All pieces of the apparatus must be thoroughly dry, as must the sample and the solvent. Oxygen must be excluded from the apparatus. Temperatures should be observed and corrections applied to burette readings, since a degree change in temperature causes variation of about 0.08 ml in the burette reading; and, if 4 ml of methane was collected, this change in temperature would cause a 2% error.

The Grignard reagent as stored for the above procedure is stable for a month or more, provided the stopper is air-tight.

The following compounds were used to test this procedure for determining active hydrogen and for determining compounds which add Grignard reagent: aniline, water, acetophenone, amyl alcohol, *tert*-butylphenol, benzaldehyde, and salicylaldehyde.

7 Acetylenic Hydrogen (HC≡CR)

Hydrogen atoms adjacent to the triple bond of an acetylenic compound are easily replaced by silver, cuprous or mercurous atoms; the metallic derivatives are generally insoluble in water. This reaction is the basis for the methods of analysis described below. One method employs silver nitrate. An excess of silver nitrate is added to the sample, the precipitate is removed, and the excess silver is titrated; or the nitric acid liberated by the reaction can be measured.

$$AgNO_3 + HC \equiv CR \rightarrow AgC \equiv CR \cdot AgNO_3 + HNO_3$$

The second method uses cuprous chloride, and the hydrochloric acid liberated by the reaction is titrated.

$$CuCl + HC \equiv CR \rightarrow CuC \equiv CR + HCl$$

The silver method has been modified to handle the determination of gaseous acetylenic compounds. The cuprous method is less accurate than the silver methods, but it is applicable to samples with which silver cannot be used because of interfering reactions.

Three silver methods are described in this section. Method A consists of reactions of ammoniacal silver solution, in which the excess silver is determined. Method B employs alcoholic silver nitrate followed by titration of the nitric acid formed in the reaction. This method is especially useful for water-insoluble samples but can also be used for water-soluble samples. Method C, which is similar to method A, is applicable to samples which contain aldehydes; aldehydes are serious interferences in methods A and B because they reduce the silver ions present.

SILVER METHODS

Method A

The above reaction will not take place if a solution of silver nitrate as such is added to the acetylenic compound. An alkaline material such as ammonia has to be present to remove the nitric acid formed in the reaction. Without the ammonia, the reaction

would take place only to a slight degree, if at all, since acids react with the silver acetylides to liberate the free acetylenic compound.

All the acetylenic compounds tried quantitatively add one more silver atom than there are acetylenic hydrogen atoms. This has also been found to be true by other investigators.[1] This method is reproducible to about ±0.5%.

Reagents.

0.1 *N* standard silver nitrate.
0.1 *N* ammonium thiocyanate.
C.p. conc. ammonium hydroxide.
C.p. conc. nitric acid.
Approx. 6 *N* sodium hydroxide.
Approx. 1 *M* ammonium hydroxide.
Saturated ferric alum indicator solution.

Procedure. Into a 250-ml glass-stoppered Erlenmeyer flask 25 ml of standard 0.1 *N* silver nitrate is pipetted. Then 3 ml of 6 *N* sodium hydroxide is added, followed by concentrated ammonia until the precipitate just dissolves; then 2 ml of excess ammonia is added. A sample containing about 0.0015 equivalent of acetylenic hydrogen is weighed in a glass ampoule (see Section 25) and placed in the Erlenmeyer flask, together with the ammoniacal silver nitrate. Some glass beads are added to aid in breaking the ampoule. The stopper should be greased if the sample is very volatile. The flask is stoppered and shaken to break the ampoule. The shaking is continued for 3 to 5 minutes to assure complete reaction. The flask is opened and the contents are quantitatively transferred to a 50-ml centrifuge tube. The flask should be rinsed three times with 3-ml portions of 1 *N* ammonium hydroxide; the washings are added to the centrifuge tube. Not all the precipitate need be removed from the flask. The mixture is then centrifuged and the supernatant liquid is carefully decanted into a 250-ml Erlenmeyer flask, care being taken not to get any of the precipitate into the flask. If particles of the precipitate persist in remaining at the surface of the liquid, the liquid can be decanted into the flask through a funnel containing a plug of glass wool. The precipitate in the funnel should not be allowed to dry for the reason given below in Cautions, p. 53. The contents of the centrifuge tube

[1] For instance, V. J. Altieri, *Gas Analysis and Testing of Gaseous Materials*, p. 329, American Gas Association, Inc., New York, 1945.

are washed three times with about 10-ml portions of 1 N ammonium hydroxide, with centrifuging each time. The washings are added to the flask with the filtrate, care being taken not to get any particles of precipitate into the filtrate.

The filtrate is then carefully neutralized with concentrated nitric acid and a few milliliters of excess nitric acid are added. The solution is then titrated with standard 0.1 N ammonium thiocyanate, ferric alum indicator being used.

Calculations.

$$\frac{\text{Milliliters } NH_4CNS \times N\ NH_4CNS \times \text{mol wt. compound} \times 100}{\text{Grams sample} \times 1000 \times B} = \% \text{ acetylenic compound}$$

where B = number of acetylenic hydrogen atoms per molecule plus one (see equation, p. 49).

The above procedure was tested with propargyl alcohol and solutions of acetylene in methyl vinyl ether. Aldehydes or other materials which reduce silver ion will interfere. Cyanides will interfere because of complex ion formation and sulfides will interfere because of formation of the insoluble silver sulfides.

Gaseous Samples

In gas samples in which acetylene or any gaseous acetylenic compound is to be determined, the method is essentially the same as the silver method described above except that a suitable apparatus is needed. In most cases, an ordinary Orsat gas analysis apparatus can be used for these analyses. However, the method described here has a much higher degree of accuracy and is capable of determining much smaller amounts of acetylenic compound than the Orsat apparatus. This procedure is accurate and precise to $\pm 0.5\%$.

Reagents.

0.1 N aqueous standard silver nitrate:
0.05 N standard ammonium thiocyanate.
Conc: ammonium hydroxide.
6 N sodium hydroxide.
Conc. nitric acid.
Saturated ferric alum indicator.

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Apparatus.

(See Fig. 8.)

Procedure. The gas sample is introduced into the 100-ml gas burette *A* through *B*. In a 50-ml volumetric flask 25 ml of 0.1 *N* standard silver nitrate is placed. To this is added 3 ml of approximately 6 *N* sodium hydroxide, followed by concentrated ammonium hydroxide, until the silver oxide precipitate just dissolves. The solution is then diluted to the mark. Into receiver

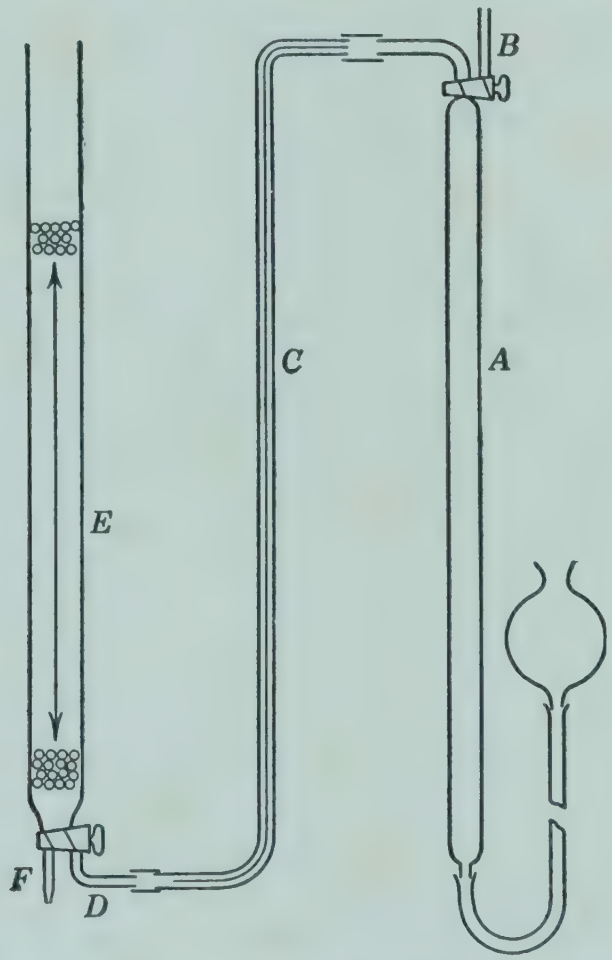


FIGURE 8. Acetylenic hydrogen apparatus for gaseous samples.

E is pipetted 25 ml of this solution. Receiver *E* is a tube of about 1.5 cm diameter with a plug of glass wool at the bottom and packed two-thirds full with 6-mm glass beads.

The gas sample is allowed to stand about 10 minutes in the gas burette for thermal equilibrium to be established and, then the volume and the temperature are read. The leveling bulb is raised, putting the sample under about 10 cm pressure, and the stopcock on the gas burette is turned to allow the gas in the burette to escape through *C*. Then the stopcock on the receiver is opened

to permit the gas into the receiver. The rate of flow of sample should be about one bubble per second. The mercury level is raised as the gas flows out of the gas burette. The mercury is forced over through *C* and *D* until it just appears in the receiver. This is to insure sweeping all the gas sample into the receiver. The contents of the receiver are then drained out by means of *F* into a 250-ml Erlenmeyer flask through a funnel with a glass wool plug to catch any silver acetylide particles which might come out of the receiver. There should be no particles of the silver acetylide in the Erlenmeyer flask. The contents of the receiver should be transferred quantitatively by rinsing with water and adding the washings to the Erlenmeyer flask through the funnel. The contents of the flask are acidified with concentrated nitric acid and a few milliliters of nitric acid are added in excess. The solution is then titrated with 0.05 *N* standard ammonium thiocyanate solution, ferric alum indicator being used.

CAUTION

(1) Silver acetylides are explosive when dry. All pieces of apparatus which contain silver acetylides should be rinsed in nitric acid to dissolve the acetylides and then rinsed with water. The receiver in the above analysis should be cleaned in the same manner, and then, if the apparatus is not in use, the receiver should be filled with dilute nitric acid in case there should be some undissolved silver acetylide particles between the glass beads or on the glass wool.

(2) Ammoniacal silver nitrate, when allowed to dry, can form explosive compounds. Therefore, all excess ammoniacal silver nitrate should be disposed of when not in use.

Calculations.

$$\text{Milliliters } AgNO_3 \text{ (ammon.)} \times N \text{ } AgNO_3 \text{ (ammon.)} = A \times N \text{ } NH_4CNS$$

A – milliliters NH_4CNS used in titration = Milliliters NH_4CNS equivalent to Ag consumed by acetylenic compound = B

$$\frac{B \times N \text{ } NH_4CNS \times \text{mol. wt. compound}}{1000C} = \text{Grams acetylenic compound} = D$$

C = number of silver atoms added to the acetylenic compound plus 1 (see equation, p. 49)

In the case of solid or liquid samples,

$$\frac{D \times 100}{\text{Grams sample}} = \% \text{ acetylenic compound}$$

To obtain the percentage of acetylenic compound in a gaseous sample, the density of the sample should be known at a certain temperature, in which case the volume of sample is converted to the volume at the temperature at which the density is known, by means of the equation $PV/T = P_1V_1/T_1$. Then, if the density is known, the volume of sample is converted to grams of sample, and this value is substituted in the above equation to calculate percentage of acetylenic compound.

If the density of the sample is not known, the result can be obtained in terms of volume percentage. The grams of acetylenic compound (D) are converted to volume by using the density (obtainable in chemical or engineering handbook) and

$$\frac{\text{Volume acetylenic compound} \times 100}{\text{Volume sample}} = \% \text{ acetylenic compound by volume}$$

The above procedure was tested with gaseous mixtures of acetylene, air, and carbon dioxide. It was found to be accurate to $\pm 0.5\%$. The interfering substances in this procedure are the same as those in the preceding procedure.

Method B

This method involves the reaction of the acetylenic compound with silver nitrate, the solution being alcoholic to deal with water-insoluble samples. The acid liberated by the reaction is titrated with standard alkali:

$$HC{\equiv}CR + 2AgNO_3 \rightarrow AgC{\equiv}CR \cdot AgNO_3 + HNO_3$$

Method of The Texas Company

(As described by V. J. Altieri, *Gas Analysis and Testing of Gaseous Materials*, American Gas Association, Inc., New York, pp. 330–332, 1945. Adapted with permission of the American Gas Association, Mr. Altieri, and the Texas Company.)

Reagents.

0.02 *N* sodium hydroxide for samples containing less than 1% acetylenic compound.

0.1 *N* sodium hydroxide for samples containing more than 1% acetylenic compound.

Silver nitrate solution: 100 g of silver nitrate dissolved in water and diluted to 1 liter (store in dark bottle). For the working solution, 35 ml of the aqueous silver nitrate solution is diluted to 140 ml with 95% ethanol.

Indicator: alcoholic solution composed of a mixture of 0.1% methyl red and 0.05% methylene blue. Store in a dark dropping bottle.

Procedure. In a 250-ml Erlenmeyer flask 50 ml of silver nitrate solution is placed together with the sample, which should contain 0.002 equivalent of acetylenic hydrogen if the 0.1 *N* sodium hydroxide is to be used in the final titration; or 0.0004 equivalent of acetylenic compound if the 0.02 *N* sodium hydroxide is to be used.

About 6 drops of indicator are added, and the contents of the flask are titrated with the sodium hydroxide solution of appropriate strength. The endpoint is the change of the red color of the indicator to a dull yellow.

For gaseous samples, the procedure described on pp. 51–54 can be used, except that the silver nitrate can be put in the receiver and the ultimate analysis made as described above instead of determining excess silver.

The above procedure is accurate and precise to ±0.5 to 1.0%. It was tested with 3-butyne-1-ol; ethynyl-cyclohexanol, 1-hexyne and 3-butyne-2-ol. Any acidic or basic materials in the sample will interfere; they should be determined on a separate sample, and the proper correction applied to the acetylenic hydrogen result. Aldehydes or other reducing substances interfere by reducing the silver ions to metallic silver, which darkens the solution to such a degree as to completely obscure the endpoint. Also, the acids formed on the oxidation of the aldehyde would interfere even if the metallic silver formed did not obscure the endpoint.

CAUTION

Silver salts of acetylenic compounds are explosive when dry. Destroy these salts by dissolving in dilute nitric acid; or by making the solution ammoniacal and dissolving the silver acetylide with 5% potassium cyanide (Hood). The resulting solution is poured into 5% ferrous sulfate to destroy the excess cyanide.

Method C

The following procedure is of service when aldehydes are present with the acetylenic compound. In the two preceding methods aldehydes interfere seriously because of their reducing action on silver ions. This procedure employs sodium acetate instead of ammonia to remove the nitric acid formed by the reaction of the silver nitrate with the acetylenic compound. The aldehydes reduce the silver ion much more slowly when sodium acetate is used as the basic medium instead of ammonium hydroxide.

Reagents.

Standard 0.1 *N* silver nitrate.
Standard 0.1 *N* ammonium thiocyanate.
Sodium acetate solution 2%.
Ferric alum indicator.
Concentrated nitric acid.

Procedure. Into a 250-ml Erlenmeyer flask is introduced 50 ml of 0.1 *N* silver nitrate. To this is added 50 ml of 2% sodium acetate solution. The solution is cooled in ice and the sample, which should contain about 0.002 equivalent of acetylenic compound, is added. The resultant precipitate is filtered with suction, by using a sintered glass funnel, and is thoroughly washed with a minimum of chilled 0.01% sodium acetate. The precipitate should be covered with liquid at all times since it decomposes when exposed to air.

The filtrate is acidified with 5 to 6 ml of concentrated nitric acid and is titrated with 0.1 *N* standard ammonium thiocyanate, ferric alum indicator being used.

Large amounts of aldehydes will reduce the silver ions relatively rapidly and consequently will interfere. However, this difficulty can be surmounted by cooling the silver nitrate–sodium acetate solution thoroughly before the sample is added. Then, after the sample is added, the resultant precipitate is filtered off immediately into a filtration flask containing 5 to 6 ml of concentrated nitric acid. In this way, the filtrate is acidified as soon as it passes through the funnel and any reaction between the silver ions and the aldehydes is prevented. The filter should be as coarse as possible so as to hasten the filtration. The reaction between the aldehyde and the silver ions is sufficiently slow, especially when the solutions are cold, so that the filtration of the silver salt of

the acetylenic compound can be achieved before any significant interference of the aldehyde can be noted.

CAUTION

See previous procedures.

Calculations.

Most acetylenic compounds consume one more atom of silver than the number of acetylenic hydrogen atoms:

$$HC{\equiv}CR + 2AgNO_3 \rightarrow AgC{\equiv}CR{\cdot}AgNO_3 + HNO_3$$

Milliliters NH_4CNS equivalent to 50 ml of $AgNO_3 = A$

A − milliliters NH_4CNS used to titrate sample $= B$

$$\frac{B \times N\ NH_4CNS \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 1000 \times C} = \% \text{ acetylenic compound}$$

C = number of acetylenic hydrogen atoms plus one

Cuprous Method

This method involves reaction of cuprous chloride with the acetylenic compound in a pyridine solution. The hydrochloric acid liberated is titrated with standard alkali.

$$Cu_2Cl_2 + 2HC{\equiv}C{-}R \xrightarrow{\text{pyridine}} 2CuC{\equiv}C{-}R + 2HCl$$

Pyridine is a weak base which combines with the liberated hydrochloric acid so that the reaction will proceed to completion. The hydrochloric acid can be titrated with sodium hydroxide, a stronger base than pyridine.

This method is not so accurate or precise as the silver method described above; the reproducibility of this procedure is ±2%. However, this method can be used for samples containing impurities that interfere with the silver method.

Reagents.

Approximately 0.1 *N* cuprous chloride. In a solution of 150 ml of pyridine and 150 ml of water 10 g of cuprous chloride is dissolved. The resulting solution is diluted to a liter with distilled water. Fresh solutions should be prepared each day due to instability of the reagent toward oxygen.

0.5 *N* standard sodium hydroxide.

Procedure. A sample containing about 0.005 equivalent of acetylenic compound is dissolved in a minimum of water (pyridine can be used if the sample is insoluble in water), and 100 ml of cuprous reagent is added to the sample. A precipitate of the cuprous derivative of the acetylenic compound will result. The solution is then titrated with 0.5 *N* sodium hydroxide, by using a *p*H meter with the standard calomel and glass electrodes. An indicator cannot be used because of the intensity of color of the solution and the presence of the colored precipitate. A plot of *p*H versus milliliters of reagent will reveal the endpoint accurately. However, the *p*H change at the endpoint is large enough to be visible from the *p*H readings recorded; a plot is necessary only for higher accuracy.

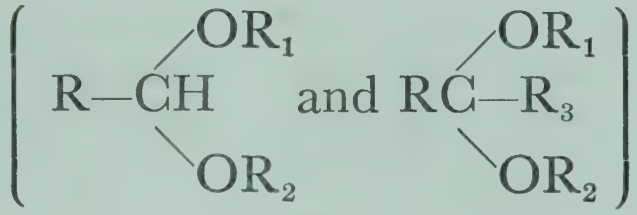
Calculations.

$$\frac{\text{Milliliters NaOH} \times N \text{ NaOH} \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 1000 \times A} = \% \text{ acetylenic compound}$$

A = number of cuprous atoms which add to the acetylenic compound

The above procedure was tested by using propargyl alcohol. Interfering substances consist of any acidic or alkaline material which may be present. Aldehydes do not interfere with this method as they do with the silver methods A and B.

8 Determination of Acetal and Ketal Type Compounds

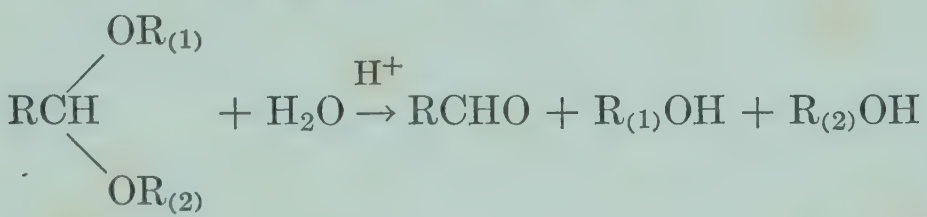


and Vinyl Alkyl Ethers ($ROCH{=}CH_2$)

ACETALS AND VINYL ALKYL ETHERS

Many of the analytical methods for acetals are connected with measurement of hydrolysis rates of acetals. The measurements are perfectly adaptable for determining rate of hydrolysis but cannot readily be used to determine absolute amounts of acetal since they are of a relative nature. Among such methods are those of Palomoa and Salomen[1] and of Tong and Olson;[2] they followed the rate of hydrolysis of acetals by dilatometer measurements.

The analysis described below involves the hydrolysis of the acetal and determination of the aldehyde formed by the bisulfite method for aldehydes as described on p. 13.



Methods for determining acetals in which the acetal was hydrolyzed and the aldehyde determined have been described. The drawbacks in these procedures are due, in most cases, to the shortcomings of the aldehyde procedure used. (These were discussed in the section on aldehydes pp. 10–16.) Also, many of the procedures require distillation of the aldehyde into the medium in which it is to be determined. This step not only greatly increases the time required for an analysis but also can be a source of error.

[1] M. H. Palomoa and A. Salomen, *Ber.*, **67B,** 424–429 (1934).

[2] L. K. Tong and A. R. Olson, *J. Am. Chem. Soc.*, **65,** 1704–1707 (1943).

Hahnel and Lennerstrand[3] avoided the distillation and used a hydroxylamine method to determine the aldehyde. The endpoints were admittedly bad, and dilutions to a specified volume for color comparisons were necessary to obtain the endpoint.

Since all the reactions involved in this analysis are carried out in the same closed vessel, there is less danger of loss of aldehyde by volatilization. The reagents used are stable, and there are no endpoint or equilibrium difficulties to harass the operator. Also there is no need for distilling the aldehyde to determine it; the aldehyde is determined in the hydrolysis reaction mixture. The procedure is accurate and precise, on the average, to $\pm 0.3\%$. This same procedure can be used to determine alkyl vinyl ethers since these compounds also hydrolyze readily to yield acetaldehyde.

$$\mathrm{ROCH{=}CH_2 + H_2O \xrightarrow{H^+} CH_3CHO + ROH}$$

Mixtures of acetals and alkyl vinyl ethers can be determined by first hydrolyzing the sample and determining the total aldehyde produced. The vinyl ether above is determined by the iodimetric procedure described on pp. 106–107. The difference between the two results will yield the amount of acetal present.

Apparatus.

(See Fig. 9.)

Adapted from the Procedure of Siggia

(*Ind. Eng. Chem.*, Anal Ed., **19.**, 1025 [1947].)

The apparatus is shown in Fig. 9. The top portion (*A*) consists of a 500-ml Erlenmeyer flask with a 29/26 standard-taper ground-glass female joint blown onto the bottom, with glass stoppers to fit. To the mouth of the flask is attached a 6-mm stopcock, and onto the stopcock is sealed a 29/26 standard-taper ground-glass male joint.

The bottom portion of the apparatus (*B*) is a 500-ml Erlenmeyer flask with a 29/26 standard-taper female joint sealed onto the mouth.

[3] Hahnel and Lennerstrand, *Svensk Kem. Tid.*, **53**, 336–351 (1941).

Reagents.

1 *N* standard sulfuric acid.
1 *N* standard sodium hydroxide.
Approximately 1 *M* sodium sulfite prepared as described on p. 14.

Procedure. A sample of 0.02 to 0.04 mole of acetal or alkyl vinyl ether is weighed in a sealed thin-walled ampoule (see Section 25) and is introduced into section *A* of the apparatus shown in Fig. 9, together with 50 ml of standard 1 *N* sulfuric acid. About 10 to 20 ml of glass beads is included to aid in breaking the ampoule. The apparatus is stoppered, the stopper being well greased to prevent leakage of any volatile aldehyde. If the aldehyde produced is acetaldehyde the flask is totally immersed in an ice-water mixture for 5 minutes to avoid building up pressure when an ampoule is broken. If the flask is not chilled, the stoppers sometimes are forced open or leaks are created in the stopcock by the pressure.

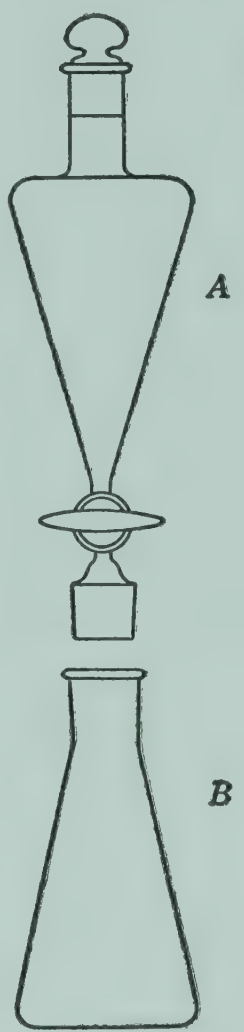


FIGURE 9. Acetal-vinyl ether apparatus.

After the flask is chilled, it is vigorously shaken to break the ampoule containing the sample; and the shaking is continued for 15 minutes to insure complete hydrolysis of the sample.

For most acetals and vinyl ethers, 15 minutes of shaking is sufficient for hydrolysis; but for some, for example, di-*n*-butyl acetal, 30 minutes is needed to insure complete hydrolysis. A Kahn shaker is used to shake the sample. This type of shaker clamps the stopper on the flask while shaking is in progress.

After hydrolysis is complete, section *A* of the apparatus, containing the hydrolyzed sample, is attached to section *B*, which contains 250 ml of 1 *M* sodium sulfite. The joint connecting the two sections should be greased to prevent loss of low boiling aldehydes. Then the stopcock in section *A* is opened, and the acid solution containing the aldehyde is shaken down into the sulfite solution.

The acid and sulfite form bisulfite, which consumes the aldehyde. After all the acid solution is in section *B*, some of it is shaken back into section *A* to react with the aldehyde contained on the walls and on the glass beads. The whole apparatus is vigorously shaken for a few minutes to assure removal of all the aldehyde vapor from the atmosphere in both sections of the apparatus.

The sections of the apparatus are then separated and rinsed quantitatively into an 800- to 1000-ml beaker, and the solution is titrated with standard 1 *N* sodium hydroxide. A *p*H meter, such as is described on p. 13 for aldehyde determinations, is used. As described on p. 14, plotting *p*H versus milliliters of sodium hydroxide is the most accurate method of determining the endpoint. However, if a large enough sample is taken, titrating to a definite *p*H for the aldehyde does not incur any very significant error.

Any free aldehyde in the sample is determined as on pp. 13–16, and the value is subtracted from the figure obtained for the acetal.

In the determination of acetals, the hydrolysis step depends on the effectiveness of shaking and ease of hydrolysis of the particular acetal as far as time necessary for hydrolysis is concerned. The shaking is important, since the samples are immiscible with water; and the more vigorous the shaking, the more sample surface is in contact with acid. The procedure as described above calls for 15 minutes of shaking for complete hydrolysis. However, this time interval is used to insure complete hydrolysis for all samples with a rather leisurely shaking of the sample with the acid.

The hydrolysis can be completed in 5 minutes if the shaking is vigorous, and a good mixing of sample with the acid is maintained. If the shaking is too leisurely, or if the flask is not shaken at all, the hydrolysis may not be complete even after 15 minutes.

The nature of the sample has a great deal to do with the time required for hydrolysis. Dimethyl acetal hydrolyzes very easily. However, di-*n*-butyl acetal requires more vigorous treatment and should be shaken vigorously with the acid for 15 minutes to insure complete hydrolysis. If agitation is moderate, it should be shaken for 30 minutes.

The direct addition of the hydrolysis reaction mixture into the sulfite solution and titration of the resultant reaction mixture eliminates the time-consuming distillation of acetaldehyde used in former acetal procedures. Also, the sodium sulfite–sulfuric acid

procedure for aldehydes is preferable to other procedures (pp. 10–13). The fact that the hydrolysis and reaction of the aldehyde with the sodium sulfite–sulfuric acid mixture are carried out in one closed system eliminates the dangers of error due to loss of aldehyde. These factors lend a high degree of accuracy and precision to the procedure.

The following were used to test this procedure: dimethyl, diethyl, di-*n*-propyl, diisopropyl, di-*n*-butyl, and diisobutyl acetal and methyl, ethyl, propyl, *n*-butyl, and isobutyl vinyl ether.

ACETALS, VINYL ALKYL ETHERS, AND KETALS

The original source of this very generally used procedure cannot be traced. The method is similar to that of Bryant and Smith described on p. 17, except that water is used as the solvent.

The procedure involves hydrolysis of acetals, vinyl ethers, or ketals with hydroxylamine hydrochloride used as the acid catalyst and also as the agent to remove the resulting aldehyde or ketone by forming the oxime; this pulls the reaction to completion. The liberated hydrochloric acid (see equation p. 16) is titrated with standard alkali.

Reagents.

0.5 *M* aqueous hydroxylamine solution. The *p*H of the solution should be adjusted to 3.1 with diluted alkali solution, a *p*H meter with the standard glass and calomel electrodes being used.

Standard 0.5 *N* sodium hydroxide.

Procedure. A sample containing about 0.012 mole of acetal, vinyl ether, or ketal is weighed into a 250-ml beaker or flask containing 50 ml of hydroxylamine reagent.

For water-insoluble samples, methanol can be added to the reagent to bring about solution. However, this should be kept at a minimum because of the slowing effect it has on the hydrolysis.

The mixture is allowed to stand until the reaction is complete. Some compounds react completely in one-half hour at room temperature. Others hydrolyze so slowly that the reaction mixture has to be refluxed for some hours. Most acetals and vinyl ethers will hydrolyze in less than 2 hours at room temperature.

Ketals usually take a longer time. Chlorodimethylacetal requires 4 hours of reflux.

After hydrolysis, the reaction mixture is titrated with 0.5 *N*

sodium hydroxide, a *p*H meter with the standard calomel and glass electrodes being used. The endpoint can be detected from the recorded *p*H and milliliter data. However, if the break is too gradual, a curve of *p*H versus milliliters should be plotted. The *p*H break at the endpoint is too gradual for indicators to be used.

If much methanol has been added to dissolve the sample, at least an equal volume of water should be added before the titration is begun. This produces a sharper *p*H change at the endpoint.

If the sample is acidic or basic, it should be brought to *p*H 3.1 before it is added to the hydroxylamine reagent. The *p*H 3.1 is used since the endpoint break is obtained in the vicinity of this *p*H.

Used to test this procedure were dimethylacetal, dibutylacetal, chlorodimethylacetal, dihydroxyethylacetal; methyl, ethyl, *n*-propyl, *n*-butyl, and isobutyl vinyl ethers; 2,2-dimethoxy-1, 4-dihydroxybutane.

The procedure is usually accurate to better than $\pm 1\%$.

General Reference

K. J. Orton and P. V. McKie, *J. Chem. Soc.*, **109,** 184–186 (1916).
E. Peynaud, *Ann. fals.*, **30,** 390–400 (1937).
L. G. Riesch and M. Kilpatrick, *J. Phys. Chem.*, **39,** 561–569 (1935).
M. Ripper, *Montash.*, **21,** 1079 (1900).
Seyewetz and Bardin, *Bull soc. chim.*, **33,** 1000–1002 (1905).

9 Amino Groups

Acetylation and diazotization are the two reactions most generally used to determine amino groups directly. Acetic anhydride will react with aromatic and aliphatic primary and secondary amines to form the corresponding acetyl derivative. Diazotization can generally be employed to determine only aromatic primary amines. However, the nitrosation mixture used in diazotizing primary aromatic amines is also capable of reacting with secondary and tertiary amines (aliphatic and aromatic) to form the corresponding nitroso compounds. With a few primary aliphatic amines the nitrosation mixture yields the corresponding alcohol and nitrogen.

Some amines are basic enough to be titrated directly with standard acid. Many aliphatic amines are of this nature, but most aromatic amines are not.

Some primary, secondary, and tertiary amines can be determined by coupling (see coupling procedures, p. 115) and bromination (see Bromination, p. 111).

The acetylation procedures are the most precise and accurate, but primary and secondary, alcohols and phenols interfere, since they also acetylate. There are two methods of carrying out analysis with acetic anhydride. One is the procedure of Ogg, Willits, and Porter which has already been described in the section dealing with hydroxyl groups (pp. 3–7). This method consists of acetylating the amine, hydrolyzing the excess anhydride, and titrating the acetic acid formed. The other acetylation procedure is described by Mitchell, Hawkins, and Smith. In this method, after acetylation of the amine, a known amount of water is added to hydrolyze the remaining anhydride, and the excess water is titrated by using Karl Fischer reagent.

The diazotization procedures can be applied whenever the acetylation procedures cannot be used, for such reasons as the presence of alcohols or that amine is in water solution, in which case the anhydride reagent is useless. These procedures are usually not accurate and precise to greater than $\pm 1\%$ because of the chance

for loss of nitrous acid in the form of oxides of nitrogen on addition of the sodium nitrite to the acid solution of the amine. Also, the endpoint is not so sharp as is necessary for very accurate analysis since the reaction rate is not very rapid, especially at the endpoint. However, the method is definitely useful and is very generally applicable. This reaction is generally limited to primary and secondary amines, although it can be used to determine a few tertiary amines by nitrosation.

A method for determining primary amines has also been published which consists of the reaction of primary amines with benzaldehyde.

$$RNH_2 + C_6H_5CHO \rightarrow RN{=}CHC_6H_5 + H_2O$$

The water is determined by Karl Fischer reagent. When combined with acetylation procedures, this method makes possible the determination of both primary and secondary amines in a mixture. Also, whenever a total base titration is included, the primary, secondary, and tertiary amine content of mixtures can be determined.

ACETYLATION PROCEDURES

(1) $RNH_2 + CH_3C(=O)OC(=O)CH_3 \rightarrow R{-}N(H){-}C(=O){-}CH_3 + CH_3COOH$

(2) $R_1R_2NH + CH_3C(=O)OC(=O)CH_3 \rightarrow R_1R_2NC(=O)CH_3 + CH_3COOH$

Adapted from the Method of Ogg, Willits, and Porter

(See Hydroxyl groups, p. 4.)

Although these authors did not use their acetylation method on amines, they suggested the possibility of its use. The method has been checked and was found to operate well for primary and secondary amines.

Adapted from the Method of J. Mitchell, W. Hawkins, and D. M. Smith

(*J. Am. Chem. Soc.*, **66**, 782–784 [1944].)

Reagents.

Acetylation reagent: 142 ml of pure acetic anhydride diluted to a liter with dry pyridine.

Hydrolysis reagent: 100 g of c.p. sodium iodide and 22 ml water diluted to a liter with pyridine.

Karl Fischer reagent: In a mixture of 269 ml of dry pyridine and 667 ml of dry methanol, 84.7 g of resublimed iodine is dissolved. The solution is cooled in chopped ice and 64 g of sulfur dioxide is added, either by careful addition in liquid form or by bubbling sulfur dioxide through the cooled solution until a 64-g increase in weight has been noticed. The water content of the reagents should not exceed 0.1% since the presence of 6 g of water per liter will completely exhaust the reagent. The solution deteriorates rapidly during the first week but more slowly thereafter. Nevertheless, it should be restandardized each day.

The reagent is standardized by using a standard water solution. Eighteen milliliters of water is accurately weighed into a 1-liter volumetric flask. This is diluted to the mark with dry methanol. Several blanks are run on 10-ml portions of the methanol to ascertain the water contained therein. Then 10-ml samples of the solution of water in methanol are titrated. The endpoint is the point at which the brown iodine color persists. Subtraction of the blank titration on the methanol from the titration on the water solution will yield the titration for the amount of water added. The titer can be expressed in grams of water per milliliter of reagent.

Procedure. A sample containing about 0.010 equivalent of primary or secondary amine is weighed into a 250-ml glass-stoppered Erlenmeyer or volumetric flask. To this is added exactly 20 ml of acetylating reagent. The flask, together with a blank determination, is stoppered, shaken, and allowed to stand 30 minutes at room temperature. Then 25 ml (by way of pipette) of hydrolysis reagent is added and the flask is placed in a bath held at 60° ± 1°C. The stopper is loosened from time to time to release the expanding air. Then the flask is firmly stoppered and kept at 60°C for 30 minutes. After cooling to room temperature

the contents of the flask are titrated directly with Karl Fischer reagent to the persistence of the brown iodine color.

Total anhydride added is equivalent to the net water consumed during hydrolysis of the blank. This figure is arrived at by subtraction of the water found from the total water added before hydrolysis. Excess anhydride after acetylation is determined by subtracting the water found from the total water added to the sample.

Alcohols (except tertiary alcohols) and most phenols will interfere with this procedure since they also acetylate. However, amides, urethans, nitriles, and tertiary amines will not interfere.

Amines which have been used to test both of the acetylation procedures are: *n*-butylamine, propylenediamine, hexamethylenediamine, cyclohexylamine, benzylamine, aniline, *p*-toluidine, *p*-phenylenediamine, *p*-bromoaniline, 2-naphthylamine, *o*-aminodiphenyl, 2-aminopyridene, N-ethylaniline, 1-naphthylamine, and *p*-aminophenol.

The Ogg, Willits, and Porter method is the simpler of the two acetylation procedures to carry out. In this method the excess anhydride is hydrolyzed and the acid is titrated with alkali. The endpoint is very sharp, and there are no special precautions to observe and no special reagents are needed.

The Mitchell, Hawkins, and Smith method is more complex in that a standard water solution is needed together with the standard Karl Fischer reagent. Karl Fischer reagent is difficult to prepare; it must be standardized each day, and all solvents used must be free of water. The amount of water in the samples also has to be known.

PRIMARY AMINES

Adapted from the Method for Primary Amines

(W. Hawkins, D. M. Smith, and J. Mitchell, *J. Am. Chem. Soc.*, **66,** 1662–1663 [1944].)

$$RNH_2 + C_6H_5CHO \rightarrow RN{=}CHC_6H_5 + H_2O$$

Reagents.

Karl Fischer reagent as described in the preceding procedure
Benzaldehyde.
Sodium cyanide.
Six per cent hydrogen cyanide solution in dry pyridine.

Procedure. A sample containing up to 0.1 equivalent of primary amine is weighed into a 100-ml volumetric flask and diluted to the mark with pyridine. A 10-ml aliquot is transferred to a 250-ml glass-stoppered volumetric or Erlenmeyer flask. After 3 ml of benzaldehyde is added, the flask is stoppered, and, together with a blank, is placed in a bath held at 60° ± 1°C.

After 30 minutes the flasks are removed and cooled to room temperature. The flasks are placed in a well-ventilated hood, and 0.2 g of sodium cyanide together with 30 ml of 6% hydrogen cyanide in dry pyridine are added. The flasks are shaken vigorously for one minute and then set aside in the hood for 45 minutes. At the end of this time, the mixture is titrated with Karl Fischer reagent to the persistence of the iodine color.

Free water in the sample is best determined in acetic acid solution.

The water found, after correction for that present in the blank and in the sample, is equivalent to primary amine.

Interfering materials in this procedure are secondary amino alcohols such as diethanolamine. They react almost quantitatively with the benzaldehyde. Heterocyclic amines also interfere, reacting with benzaldehyde to form N,N′-benzaldiamines.

This procedure is very useful for determining primary amines in a mixture of primary, secondary, and tertiary amines. Combining results from this analysis with an acetylation analysis which yields primary and secondary amines, a value for secondary amines can be obtained by difference. If the primary, secondary, and tertiary amines are of sufficient basic strength to be titrated with acid, it would be possible to obtain a figure for tertiary amines in the mixture.

AMINES VIA ACIDIMETRIC TITRATION

Some amines are basic enough to be titrated with standard acid. The aliphatic amines are mostly of this type. It is not necessary to give a detailed description of a procedure since it is so simple. The best general method is to titrate potentiometrically a weighed sample with 1 *N* acid. In this way the rather strong acid will give the sharpest possible break in *p*H that can be obtained with the amine. Also, since the *p*H at which the amine is completely neutralized is not known, no indicator can be used and a potentiometric titration is the only safe method. However, once the

*p*H at the endpoint is established for a certain amine and the change in *p*H at the endpoint is sufficiently definite to give a sharp indicator change, an appropriate indicator can be selected for that particular amine.

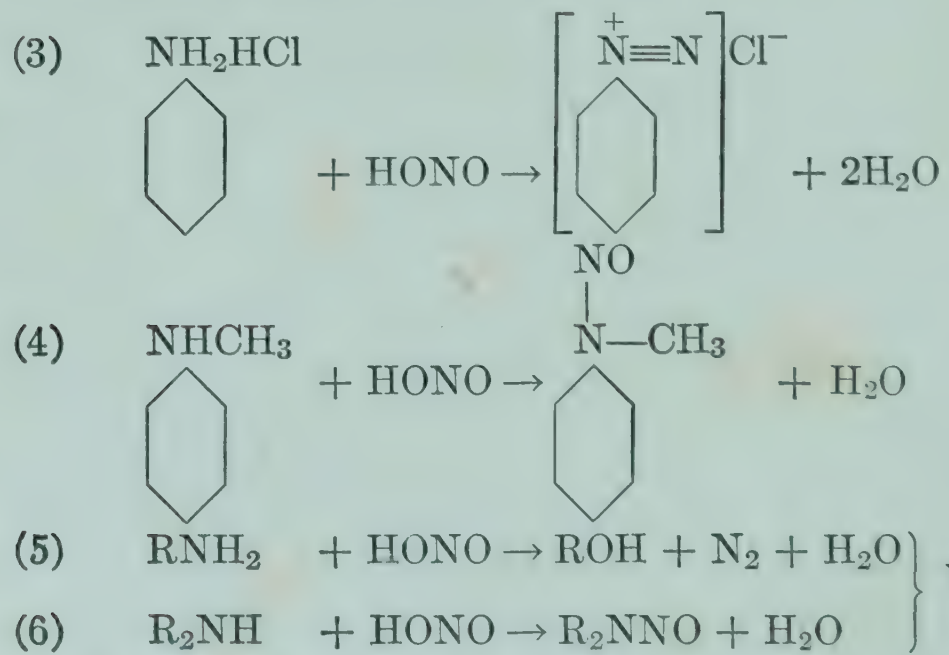
S. Palit[1] has described solvent mixtures in which sharper endpoints can be obtained when titrating aromatic and many other weakly basic amines than can be obtained in water alone. This method is described on pp. 21–22 for the determination of carboxylic acid salts. Few examples are given, but the method is generally applicable.

This method is by far the simplest way to determine amines; however, its usefulness is limited, since only a small percentage of all amines are basic enough to be titrated. Acidic or basic impurities of course interfere unless they give a separate break in the curve for *p*H versus milliliters of standard acid.

DIAZOTIZATION AND NITROSATION PROCEDURES

These procedures are those most generally used to determine amines. No specific reference can be given for any general method of employing nitrous acid. The following procedure is a conglomeration of many known procedures.

The procedure will mainly determine primary and secondary amino groups (see reactions 3 to 6). However, some tertiary amines will react with nitrous acid to form the nitrite salt but these usually cannot be determined.



[1] *Ind. Eng. Chem.*, Anal. Ed., **18**, 246–251 (1946).

Reaction 5 proceeds as written only when R is methyl or ethyl. When R is a higher alkyl group the reaction does often proceed quantitatively, but the end product is not the alcohol expected. For instance, if propyl amine is reacted with nitrous acid, both *n*-propyl and isopropyl alcohol result.

Reagents.

1 *N*, 0.5 *N*, 0.1 *N* sodium nitrite.
Starch iodide paper.
Concentrated hydrochloric acid.
Glacial acetic acid.
25% potassium bromide solution.

Procedure. A sample of amine large enough to give a 20-ml titration with nitrite of a certain strength is weighed into a 1-liter beaker and dissolved in about 500 ml of water, 30 ml of concentrated hydrochloric acid, and 25 or 50 ml of 25% potassium bromide. (The amount of potassium bromide used depends on the ease of nitrosation of the amine.) Potassium bromide can be eliminated if the amine reacts rapidly. If the sample is not soluble in dilute acid, the sample can be dissolved in a few milliliters of glacial acetic acid and then the water and hydrochloric acid are added. The amine, in such cases, generally remains in solution.

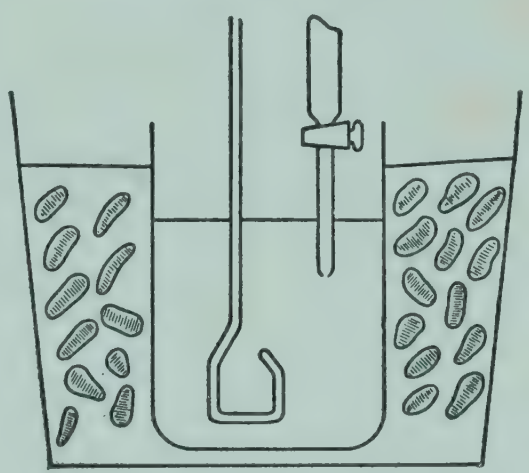


FIGURE 10.

The required strength of sodium nitrite depends on several factors: ease of nitrosation, clarity of endpoint, instability of the nitrous acid. The 1 *N* sodium nitrite will give a better endpoint, but the danger of losing nitrous acid is much greater since more is formed on each addition than is formed with the more dilute sodium nitrite. When the more dilute sodium nitrite is used, a poorer endpoint results, but the chances of loss of nitrous acid are lower.

The beaker containing the sample is immersed to within an inch of the rim in chopped ice and water until the temperature is about 5°C. This is to lower chances for loss of nitrous acid. Then with the tip of the burette well under the surface of the solution (see Fig. 10), the nitrite standard solution is added at a rate depending

on how rapidly the amine consumes the nitrous acid. There should never be a large excess of nitrite present, since this causes loss of nitrous acid. At first the nitrite should be added in small increments, and the solution tested by dipping into the solution a strip of starch-iodide paper; the paper turns the blue-black color of iodine in starch if nitrous acid is present. If the amine consumes the nitrous acid rapidly, the nitrite can be added more rapidly. If the amine nitrosates slowly, the nitrite is added more slowly. The purpose of the potassium bromide is to catalyze the nitrosation; it should be added whenever the nitrite is consumed too slowly. If the amine nitrosates too slowly, the endpoint is very hard to detect. The nitrite is added in smaller increments as the endpoint is approached. This decreases the chance of overtitration.

The endpoint is the point at which the blue-black color is produced on the starch-iodide paper when the solution has stood for quite a time after the addition of nitrite. As the endpoint is approached the nitrite will be consumed more slowly so that a sufficient length of time (depending on the rate at which the particular amine nitrosates) should be allowed before testing for the endpoint. An excess of nitrite gives the blue-black color on the paper immediately on immersion in the solution. In some cases, the starch-iodide paper, on being dipped into the solution, does not darken immediately but darkens slowly on exposure to air. This should not be construed as indicating excess nitrite since the nitrite produces the color immediately. Colored amines and precipitates will hamper the viewing of any color on the paper. However, the presence of excess nitrite still can be shown by the solution that diffuses up the indicator paper, showing a blue-black portion above the mark which indicates the depth of the immersion of the paper in the solution.

The sodium nitrite is standardized by reaction with sulfanilic acid, which can be purchased in a pure state. The procedure is that described above.

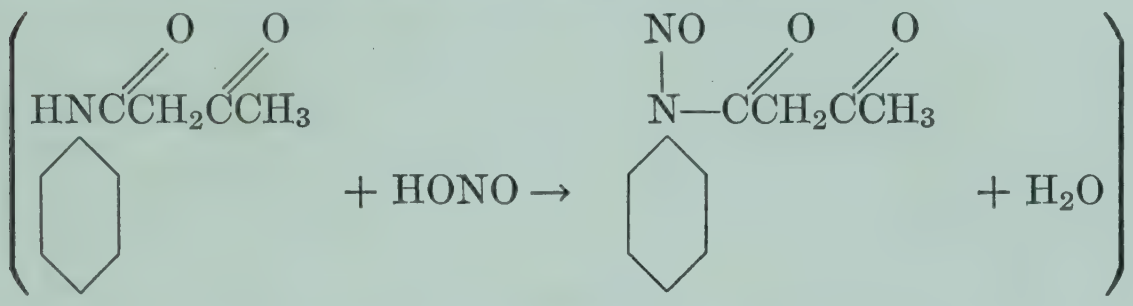
Calculations.

$$\frac{\text{Milliliters nitrite} \times N\ NO_2^- \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 1000 \times n} = \% \text{ compound based on diazotization or nitrosation}$$

n = number of amino groups per molecule

Amines tested were: aniline, 4 nitroaniline; *p*-toluidine; 1-amino-2-naphthol-4-sulfonic acid; 2,6-toluylene diamine-4-sulfonic acid; 4-aminoazobenzene; 1-amino-8-naphthol-3-6-disulfonic acid; benzidine; 4-4′-diaminodiphenylamine-2′-sulfonic acid; 4-aminophenol; 1-naphthylamine; *o*-aminodiphenyl.

Secondary amines tested were acetoacetanilide



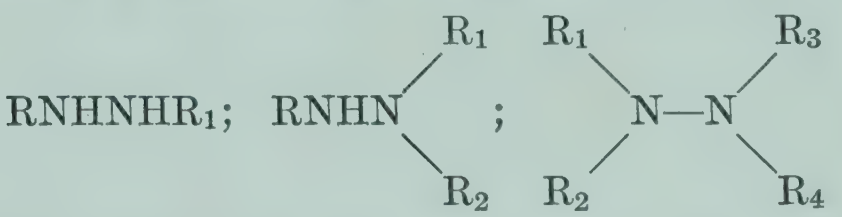
N-ethyl-1-naphthylamine, diphenylamine, N-methyl- and N-ethyl-aniline.

Interfering substances consist mainly of compounds which are *readily* reduced by nitrous acid and also compounds which contain active methylene groups. The compounds with active methylene groups will nitrosate and can be determined by this procedure. The pyrazolones are such compounds.

10 Hydrazines

Hydrazines are readily determined because they may be relatively easily oxidized and reduced. They can be oxidized by means of bromine, iodine, and copper sulfate; and reduced by titanous chloride. Generally speaking, only aliphatic hydrazines of type $RNHNH_2$ (where R is hydrogen or a small alkyl group) can be determined by means of iodine. This is largely due to the solubility of this type of hydrazine in water or bicarbonate solution, the medium in which the iodine methods are best carried out. If the group is aromatic or is relatively large, the bromine or iodine methods will not work.

If the hydrazine is substituted on both nitrogen atoms, these compounds being referred to as hydrazo compounds,



the oxidation methods are applicable only in very specific cases. In the case of the hydrazo compounds the titanous chloride reduction method (pp. 82–84) can generally be applied when the R groups are both aliphatic and aromatic.

$$(1) \qquad R_{(1)}NHNHR_{(2)} \xrightarrow{TiCl_3} R_{(1)}NH_2 + H_2NR_{(2)}$$

Aromatic hydrazines cannot generally be determined by bromine or iodine oxidation because they are soluble only in rather acid solutions, and the oxidation is slow and uncertain in such strong acid solutions. If the aromatic hydrazine contains an acidic group which makes it soluble in sodium bicarbonate solution, it usually can be determined by the iodine method. A rather general method for aromatic hydrazines, or hydrazines where the R group is a large alkyl group, is the oxidation using cupric sulfate. In these cases the nitrogen liberated by the reaction is measured. Aromatic hydrazines oxidize to the corresponding diazonium compound which decomposes with evolution of nitrogen when heated.

BROMINE OXIDATION

See Bromination procedure, p. 111.

IODINE OXIDATION

This procedure uses iodine instead of bromine for oxidizing the hydrazine.

(2) $$NH_2NH_2 \cdot H_2SO_4 + 2I_2 + 6NaHCO_3 \rightarrow 4NaI + Na_2SO_4 + 6CO_2 + 6H_2O + N_2$$

(3) $$RNHNH_2 \cdot H_2SO_4 + 2I_2 + 5NaHCO_3 \rightarrow RI + 3NaI + Na_2SO_4 + 5CO_2 + 5H_2O + N_2$$

Method Described by Joseph Rosin

(Adapted with permission from *Reagent Chemicals and Standards*, p. 194, D. Van Nostrand Co., New York, 1939.)

Reagents.

0.1 *N* standard iodine.
Sodium bicarbonate.
Starch indicator.

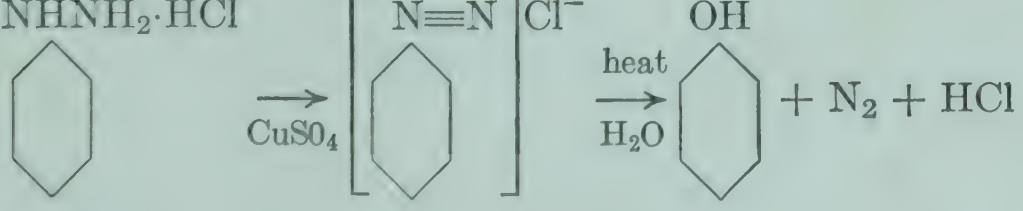
Procedure. About 0.0005 to 0.001 mole of hydrazine is dissolved in about 20 to 50 ml of water. About 1 g of sodium bicarbonate is added, and the solution is titrated with 0.1 *N* iodine, starch being used as an indicator.

$$\frac{\text{Milliliters } I_2 \times N\ I_2 \times \text{mol. wt. hydrazine} \times 100}{\text{Grams sample} \times 4000} = \% \text{ hydrazine}$$

The above procedure was described only for the determination of hydrazine sulfate. It was, however, found to work rather generally for water-soluble hydrazines.

CUPRIC SULFATE OXIDATION

In this determination, the hydrazine is oxidized to the corresponding diazonium salt.

(4) 

The diazonium salt is then decomposed and the liberated nitrogen is measured. The apparatus for this determination is the same as that used in the determination of diazonium salts (Fig. 11).

Procedure. About 0.001 mole of hydrazine is weighed into the reaction flask and a boiling chip is added. The reaction flask is attached to the condenser, grease being used to make all the ground-glass joints gastight. Through the separatory funnel 40 ml of saturated copper sulfate solution is added, followed by 10 ml of concentrated sulfuric acid. The stream of carbon dioxide is begun, and heat is applied to the reaction vessel. The contents of the reaction flask is brought to boiling and kept there until the reaction is complete, which is indicated by the fact that the volume of nitrogen ceases to increase. The gas burette sometimes becomes warm during the reaction because of the hot gas bubbling through it. It should be allowed to cool to room temperature before the reading is taken, or else the temperature recorded on the thermometer will be considerably lower than the actual temperature of the gas and the 50% potassium hydroxide. This can cause serious errors. The temperature and barometric pressure are noted.

As in the procedure for determining diazonium salts, the volume is corrected for vapor pressure of the potassium hydroxide solution and the blank for the carbon dioxide (see pp. 79–81).

Calculations.

A = volume for nitrogen (corrected for vapor pressure of potassium hydroxide and the blank for the carbon dioxide at temperature T)

$$\frac{PA}{T+273} = \frac{A^1\,760}{273}$$

$$\frac{1}{22{,}400} = \frac{x}{A^1} \qquad (x = \text{moles of nitrogen collected})$$

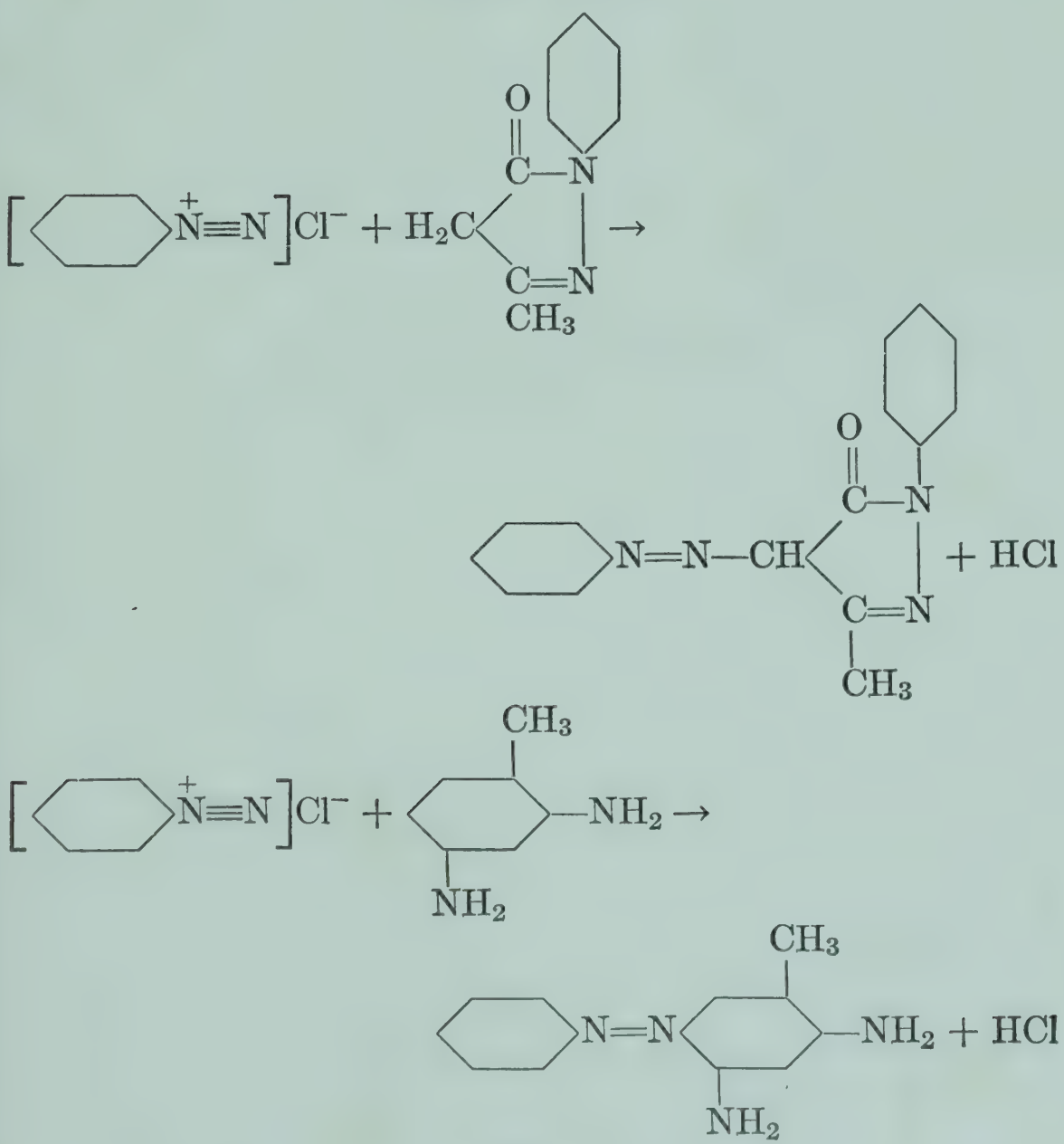
$$\frac{x \times \text{mol. wt. hydrazine} \times 100}{\text{Grams sample}} = \%\ \text{hydrazine}$$

This method is reproducible to better than $\pm 2\%$. It was tested by using phenylhydrazine, 2,4-dinitrophenylhydrazine, and tolylhydrazine. These hydrazines could not be determined by the bromine or iodine methods.

I I Diazonium Salts

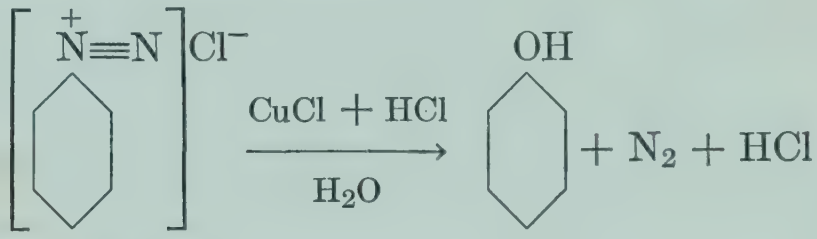
Diazonium compounds can be determined in two ways. They can be coupled with excess coupling agent (1-phenyl-3-methyl pyrazolone-5; *m*-toluylenediamine), and the excess coupling agent is then titrated with standard diazonium solution. Also, diazonium compounds can be decomposed with the liberation of nitrogen, the nitrogen is collected and measured. Both methods are equally good; the choice between them is just a question of convenience.

Coupling Method



In this method, the procedure is identical with that used to standardize the diazonium solutions in the section on coupling procedures (see p. 115). In that section an aliquot of standard coupling agent is titrated with the unknown diazonium compound as a means of standardizing the diazonium solution. In this case, the diazonium standard is known, and the ratio of coupler solution to diazonium solution is also known. An aliquot containing excess coupling agent is added to a water solution containing a weighed amount of diazonium salt. The required amount of acetic acid–sodium acetate buffer is added. The excess coupler is then titrated with standard diazonium solution. Full details can be obtained on pp. 115–120.

Nitrogen Measurement Method



Apparatus (Fig. 11).

A = 25-ml separatory funnel.
B = 100-ml nitrometer containing 50% potassium hydroxide.
C = 10-mm tubing.
D = 2-mm capillary.
G = 5-inch condenser.
H = bubbler.
I = thermometer.
J = 150-ml flask.

Reagent.

Cuprous chloride.
Concentrated hydrochloric acid.

Procedure. In about 15 ml of concentrated hydrochloric acid, 3 g of cuprous chloride is dissolved and the resultant solution is placed in the separatory funnel (*A*). A sample containing about 0.001 mole of diazonium salt is weighed into the reaction flask. The flask is attached to the condenser, all joints being greased to prevent leakage. The nitrometer (*B*) contains potassium hydroxide solution (71.5 g of potassium hydroxide per 100 ml of water) and a layer of mercury at the bottom. The level of the mercury

extends about one-half inch above the entrance tube. The function of the mercury is to prevent clogging of the capillary (*D*) with potassium carbonate. Carbon dioxide is passed through the apparatus to flush out the air; it is continuously passed through until the bubbles reach a minimum size in the nitrometer. These

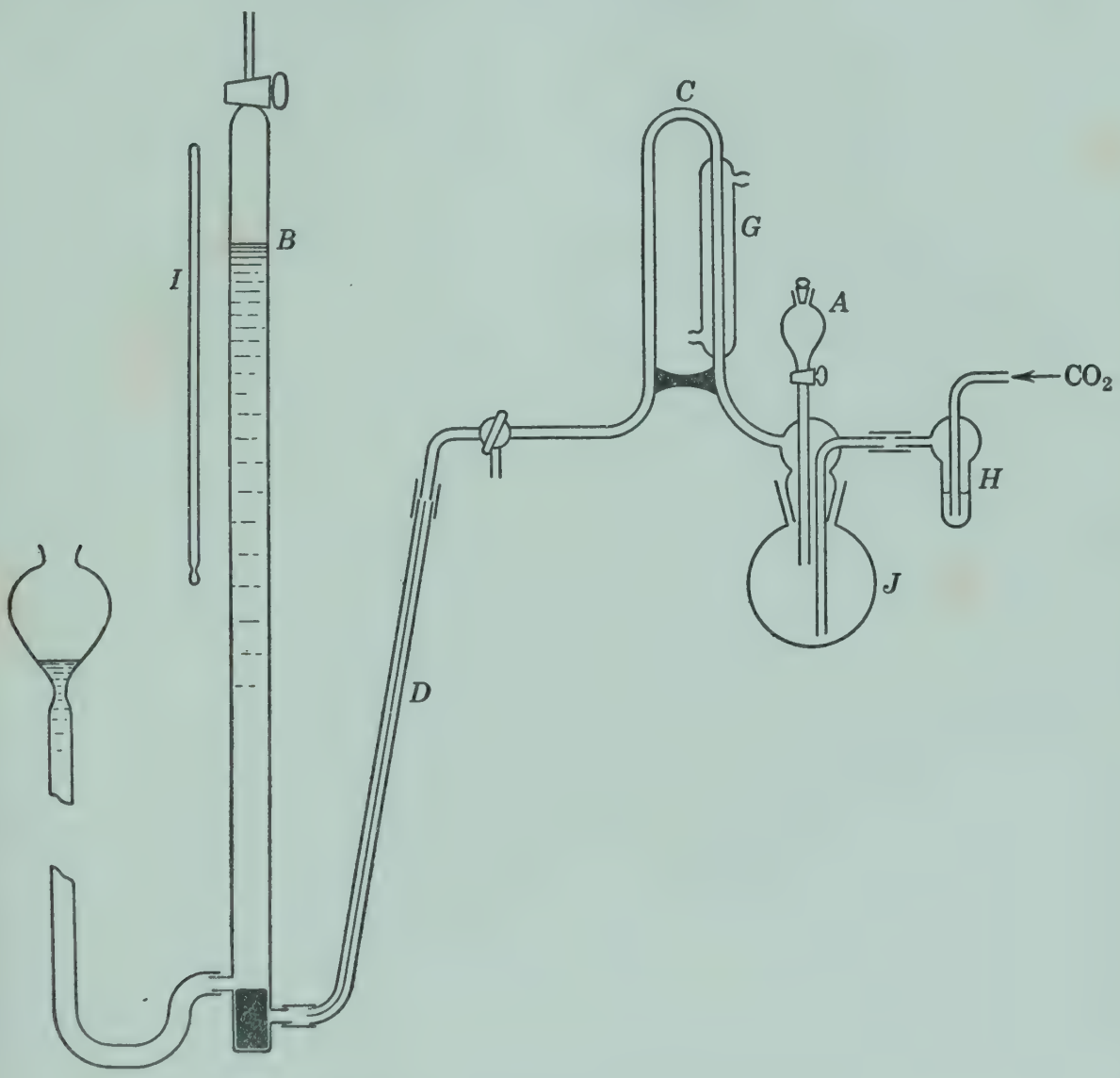


FIGURE 11: Diazo nitrogen apparatus.

minute bubbles are indicative of the potassium hydroxide in soluble impurities contained in the carbon dioxide. A blank run should be made on each cylinder of carbon dioxide to correct the volume readings for each sample. The blank determination is made as follows: After all the air is swept out of the apparatus, the accumulated inert gas is removed from the nitrometer and the number of bubbles of carbon dioxide per minute (2 bubbles per second is a convenient rate) is counted and the time is noted. Every 15 minutes the nitrometer is read to obtain the volume of inert gas

collected in the period. This is done over a period of 2 to 3 hours. The average is taken for the volume of inert gas collected per 15 minutes at that particular rate of flow. When a sample is analyzed, the carbon dioxide is fed in at the same rate as in the case of the blank. The time required for the sample to react completely is noted, the carbon dioxide blank is computed for that length of time and is subtracted from the volume recorded in the nitrometer. This correction is usually small but it is significant. Large blanks resulting from impure carbon dioxide decrease the accuracy of the results.

In an actual determination, the air is swept out of the apparatus as previously described. The inert gas is removed from the nitrometer and the solution of cuprous chloride in hydrochloric acid is allowed to flow from the separatory funnel (*A*) into the reaction flask (*J*) which contains the sample, and the time is noted. The cuprous chloride is followed by 50 to 75 ml of water, no air being allowed to enter the apparatus. The reaction flask is heated to boiling and kept at that temperature until the reaction is complete, as indicated by the bubbles in the nitrometer reaching their minimum size. The heat is shut off, and the apparatus is allowed to stand for 5 to 10 minutes to reach temperature equilibrium. The carbon dioxide flow is continued throughout this period of standing. The volume of gas collected is read, the leveling bulb on the nitrometer being used to set the pressure of the gas in the nitrometer equal to atmospheric pressure. The temperature is read on thermometer (*I*), and the barometric pressure and time are noted. The volume of gas collected is corrected for the blank determination made on the carbon dioxide, the time required for the reaction being taken into account. The volume is also corrected for the vapor pressure of the potassium hydroxide solution.

Vapor Pressure of Potassium Hydroxide Solutions Containing 71.5 Grams of Potassium Hydroxide per 100 Grams of Water

(International Critical Tables)

T°C	mm Hg
15	4.1
20	5.6
25	7.4
30	9.6
35	12.7

Calculations.

A = volume of gas, corrected for carbon dioxide blank

P = atmospheric pressure − vapor pressure of the 50% potassium hydroxide solution

T = temperature recorded at the end of analysis

$$\frac{AP}{T + 273} = \frac{A_1\ 760}{273}$$

A_1 = volume calculated to N.T.P.

$$\frac{1}{22{,}400} = \frac{x}{A_1} \qquad (x = \text{moles of nitrogen collected})$$

$$\frac{x \times \text{mol. wt. diazonium compound} \times 100}{\text{Grams sample}} = \% \text{ diazonium compound}$$

The determination is accurate and precise to better than ±1%. The apparatus is very simple to operate and to clean. The time required for each analysis varies, depending on the stability of the particular diazonium salt being determined. However, samples requiring more than one hour are rare. The following were used to test this procedure: benzenediazonium chloride; (the following compounds are zinc chloride salts) *p*-dimethylaminobenzenediazonium salt; *p*-diphenylaminediazonium salt; *p*-diethylaminobenzenediazonium salt.

This method is suitable for determining hydrazines which are first oxidized to diazonium salts and then determined as above (see pp. 74–76).

Diazonium salts are rather easily reduced. It may be possible to determine them by titanous chloride reduction (see pp. 82–84). It has not been tried, but since titanous chloride can be used to reduce azo linkages (R—N=NR$_1$), it may be possible to do the same with linkages of the type [R—N$^+$≡N] salt.

I 2 Titanous Chloride Reduction

(—N=N— ; —NO_2 ; —NHNH—)

$$RN{=}NR_1 + 4(H) \rightarrow RNH_2 + R_1NH_2$$

$$RNO_2 + 6(H) \rightarrow RNH_2 + 2H_2O$$

$$RNHNHR_1 + 2(H) \rightarrow RNH_2 + R_1NH_2$$

This procedure is readily adaptable to reduction of azo, hydrazo, and nitro groups. Indications are that this procedure may also be applicable to the reduction of diazonium compounds. Titanous ion is a strong reducing agent, and this is one of its drawbacks; atmospheric oxygen will oxidize it. The reagent must be stored under an inert atmosphere, and the reduction must be carried out under nitrogen. With proper precautions against elimination of oxygen, the procedure can be used with good accuracy and precision.

Reagents.

Titanous chloride: One liter of water containing 100 ml of concentrated hydrochloric acid is boiled to remove dissolved oxygen. This solution is cooled under nitrogen, and to it is added 560 ml of 20% titanous chloride ($TiCl_3$ is sold in 20% solution). The solution is diluted to 2 liters with freshly boiled and cooled distilled water. The solution is stored under hydrogen or oxygen-free nitrogen in an apparatus as described in Fig. 12.

Ammonium thiocyanate—10% solution.

Ferric ammonium sulfate solution: In 600 ml of water 282 g of ferrous ammonium sulfate is dissolved, and 50 ml of concentrated sulfuric acid is added. To the still warm solution, 80 to 100 ml of Perhydrol (30% hydrogen peroxide) is added slowly, with stirring. The excess peroxide is boiled off, and the solution is cooled and diluted to 4 liters. To standardize the ferric ammonium sulfate solution 20 ml of the solution is placed in a 250-ml iodine flask together with 4 ml of 4 *N* hydrochloric acid. Then 2 g of potassium iodide is added, and the solution is allowed to stand for 5 minutes. The liberated iodine is titrated with 0.1 *N* sodium thiosulfate to the starch endpoint.

Apparatus.

Storage burette for titanous chloride (Fig. 12).

The burette, as indicated in Fig. 12, is used for Karl Fischer reagent. This reagent must be protected from water vapor so that ascarite tubes are shown in the diagram. Titanous chloride solution must be protected

against oxygen so that the ascarite tubes do not apply in this case. For titanous chloride solution a hydrogen generator (a Kipp generator) is attached to the vent from the storage bulb. In this manner, a continuous hydrogen atmosphere is maintained above the solution.

Attached to the vent of the measuring burette section of the apparatus is a small stopcock.

When the apparatus is charged with titanous chloride solution, the atmosphere above the solution is purged of air by turning the burette

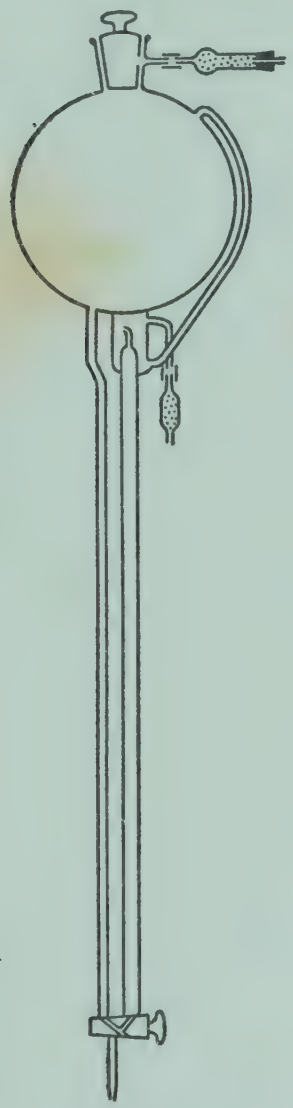


FIGURE 12.

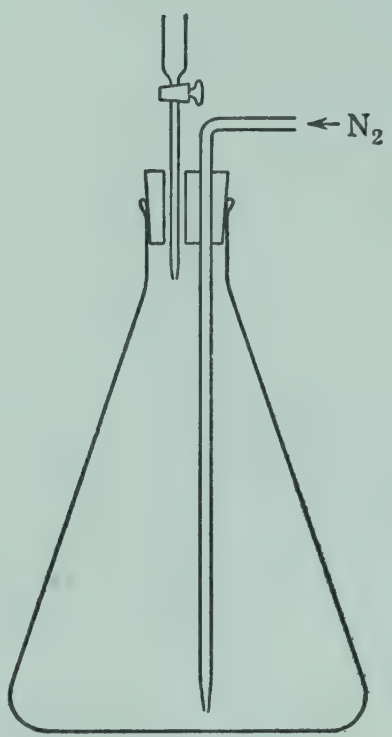


FIGURE 13.

stopcock so that the measuring burette is open to the atmosphere at the tip. The hydrogen from the generator will then flow through the by-pass tube from the reservoir, to the burette, and on to the outside through the burette tip. This operation will purge the burette as well as the atmosphere above the titanous chloride solution. In filling the measuring burette, the stopcock is turned to connect the burette to the reservoir. Then the stopcock on the vent of the burette is opened, and the burette will fill with reagent. The reagent entering the burette will force out the hydrogen through the stopcock at the vent. As soon as the burette is filled to the desired level, both the stopcock at the vent and at the tip of the burette

are closed. To introduce the reagent to the reaction vessel, the stopcock at the burette tip is opened to the reaction vessel containing the sample (through which oxygen-free nitrogen is bubbling). The hydrogen pressure in the apparatus will cause the solution to flow out of the burette.

Reaction flask (Fig. 13).

Procedure. About 0.005 equivalent of sample is placed in a 500-ml Erlenmeyer flask and dissolved in water or in glacial acetic acid if the sample is water insoluble. The flask is then equipped as shown in Fig. 13 to keep the reaction under nitrogen. Oxygen-free nitrogen is passed through the sample for 5 to 10 minutes. Next 25 ml of titanous chloride solution, 30 ml of concentrated hydrochloric acid and 2 ml of hydrofluoric acid are added. The solution is boiled for 5 minutes (a longer time may be needed for some samples). A condenser should be attached to the reaction flask for volatile samples. With the nitrogen still bubbling through the solution, the flask is cooled in ice, and 10 ml of 10% ammonium thiocyanate is added. The solution is titrated with the ferric ammonium sulfate solution to a red endpoint, while the nitrogen is kept bubbling through the solution.

A blank should be run on the titanous chloride for each set of reductions. The same solvent should be used for the blank as is used for the sample

Calculations.

$$\frac{(\text{Milliliters F.A.S. blank} - \text{milliliters F.A.S. sample}) \times N \text{ F.A.S.} \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 1000 \times A} = \% \text{ compound}$$

A = number of hydrogen atoms consumed per molecule of compound

The above procedure was used successfully to analyze 2,4-dinitrophenylhydrazine; *p*-nitraniline; 1,3,5-trinitrobenzene; benzene-azodiphenylamine; *p*-nitrobenzene-azo-resorcinol; hydrazobenzene. The average accuracy and reproducibility are better than ±2%. With special care to keep oxygen out of reagents and reaction mixture, results better than ±1% can be obtained.

Oxygen is the most troublesome interference in this procedure; however, any reducible substance will usually interfere. Other types of unsaturated compounds generally will not be reduced by titanous chloride.

13 Mercapto Groups

There are three very good methods for determining mercaptans. One method is a simple oxidation employing iodine, a second method involves reaction with cupric alkyl phthalates, and the third involves amperometric titration with silver nitrate.

The iodine method, the simplest method of the three to carry out, is very accurate. The copper method can be used when the iodine method cannot be applied because of interfering substances or inherent difficulties of the method with certain mercaptans. The silver method is the most rapid, once the apparatus is set up, and is very accurate. The method is readily adaptable to routine work. Also, the same apparatus can be used for many determinations requiring silver titrations. (For halides see Laitinen, Jennings, and Parks, *Ind. Eng. Chem.*, Anal. Ed., **18,** 355 [1946]; *ibid.*, **18,** 358 [1946]. For cyanide see *ibid.*, **18,** 574 [1946].)

Adapted from the Iodine Method of Kimball, Kramer, and Reid

(*J. Am. Chem. Soc.*, **43,** 1199–1200 [1921].)

$$2RSH + I_2 \rightarrow R{-}SSR + 2HI$$

Reagents.

0.1 *N* standard iodine:
0.1 *N* sodium thiosulfate:
Starch-indicator solution:

Procedure. A sample containing 0.002 to 0.003 equivalent of mercaptan is weighed in a sealed glass ampoule (see Section 25). The ampoule is placed in a 250-ml glass-stoppered Erlenmeyer flask with 50 ml of 0.1 *N* iodine and some glass beads to aid in breaking the ampoule. The flask is shaken vigorously to break the ampoule, and shaking is continued to assure complete reaction. The excess iodine is then titrated with 0.1 *N* standard sodium thiosulfate, starch indicator being used.

Calculations.

Milliliters of thiosulfate needed for 50 ml of the iodine used = A

Milliliters of thiosulfate used in titration = B

$A - B$ = milliliters of thiosulfate equivalent to the mercaptan present = C

$$\frac{C \times N \text{ thiosulfate} \times \text{mol. wt. mercaptan} \times 100}{\text{Grams sample} \times 1000} = \% \text{ mercaptan}$$

The following mercaptans were used to test this procedure: methyl, ethyl, propyl, *n*-butyl, *sec*-butyl, isobutyl, isoamyl, phenyl, *p*-cresyl, 2-naphthyl. Mercaptans determined by other authors were lauryl mercaptan, β-mercapto-ethanol, and terpene mercaptan. The above procedure is reproducible to ±0.4%. Hydrogen sulfide will interfere as will any material oxidized by iodine.

Adapted from the Cupric Alkyl Phthalate Method of Turk and Reid

(*Ind. Eng. Chem.*, Anal. Ed., **17**, 713–714 [1945].)

This method involves the reaction of mercaptans with the cupric ion:

$$2Cu^{++} + 4RSH \rightarrow 2CuSR + RSSR + 4H^{+}$$

Cupric butyl phthalate and cupric octyl phthalate are used as the reagents to titrate the mercaptans directly. This method is fast and accurate but is somewhat less accurate than the iodine method. This procedure is advantageous because it is effective in the presence of many substances which would be oxidized in the iodine method and thus yield erroneous results. Unsaturated compounds will also not interfere in this procedure, whereas they usually do in the iodine method. Hydrogen sulfide will interfere in this procedure because of precipitation of copper sulfide. No interference was observed by hydrogen cyanide, organic thiocyanates, organic sulfides, thiocyanoacetates, and terpenes.

Reagents.

Cupric butyl phthalate: To 50 ml of *n*-butanol contained in a 500-ml Erlenmeyer flask is added 74 g of finely divided phthalic anhydride. The mixture is heated with agitation to about 105°C. (The phthalic anhydride should not be allowed to hydrate by exposure to moisture. A sample of anhydride should remain clear when heated to 131°C in a test tube.) The heating is stopped while the agitation is continued. The temperature continues to rise to 120°C, and the mixture becomes clear in a few minutes. The solution is then cooled and poured into a solution of 20 g of sodium

hydroxide in 1500 ml of water. If the resulting solution is not acid, it should be made so with acetic acid. The solution is filtered into a 4-liter beaker. A solution of 65 g of cupric sulfate (pentahydrate) in 500 ml of water (filtered to remove any solid particles) is slowly poured into the butyl phthalate mixture with vigorous agitation. The cupric butyl phthalate which precipitates is collected on a Büchner funnel, washed with water, and dried in air. It is pulverized in a mortar and dried in a vacuum desiccator. The yield is about 95%.

The purity of the cupric butyl phthalate has to be determined since this material is used as the standard. This is done by dissolving a 0.3 to 0.5 g sample in 5 ml of glacial acetic acid, diluting with 50 ml of water, and determining the copper iodometrically. The cupric butyl phthalate is stable for very long periods of time.

Cupric octyl phthalate: This reagent is prepared exactly as the cupric butyl phthalate except that 68 g of *n*-octanol is used instead of the *n*-butanol. Cupric octyl phthalate is difficult to filter, and washing by decantation is often necessary. Cupric butyl phthalate is more easily prepared and is more generally applicable. Therefore, there is no need to use the cupric octyl phthalate.

To prepare the standard solutions, 25.29 g of the cupric butyl phthalate (or 30.9 g of cupric octyl phthalate) is used to prepare a 0.1 N solution. If P = purity of the cupric butyl phthalate, $25.29 \times 100/P$ g are required for a 0.1 N solution. The cupric butyl phthalate is weighed into a 1-liter volumetric flask, dissolved in 50 ml of acetic acid, and diluted to a liter with Pentasol, a mixture of the isomers of amyl alcohol (butanol, pentanol, or any hydrocarbon solvent can be used). The solution is standardized by the method of Weatherburn, Weatherburn and Bayley[1] which consists of pipetting a sample of solution containing about 50 to 100 mg of copper into a beaker and adding 10 ml of 1:1 hydrochloric acid. The mixture is boiled for 2 to 5 minutes, then cooled and poured into a separatory funnel. The aqueous layer is drawn off, and the organic layer is washed three times with 15 ml of water; the water washings are combined with the original water layer. If the oil and water layers tend to emulsify 3–5 ml of isopropyl alcohol can be added. The water solution is made alkaline with ammonium hydroxide and then very slightly acidic with glacial acetic acid. About 2 g of potassium iodide is added and the liberated iodine is titrated with 0.1 N thiosulfate, starch indicator being used.

The standardization should be carried out at about the same temperature as that in the procedure given below. A 10° temperature difference causes about a 0.1-ml error per 10 ml titration. The normality of the cupric $\begin{Bmatrix}\text{octyl}\\\text{butyl}\end{Bmatrix}$ phthalate can be expressed as

$$\frac{\text{Milliliters of thiosulfate} \times N \times 2}{\text{Milliliters of copper solution}} = N \text{ copper solution}$$

(The factor 2 in the numerator is due to the reaction of 2 moles of mercaptan per mole copper reagent.)

[1] *Ind. Eng. Chem.*, Anal. Ed., **16**, 703 (1944).

The cupric alkyl phthalate solutions are stored in dark bottles to retard peroxide formation. Solutions containing considerable amounts of precipitate should be discarded.

Procedure. A sample containing 0.1 to 0.3 g of mercaptan (depending on molecular weight) is weighed into a 125-ml Erlenmeyer flask containing 50 ml of Pentasol (a synthetic amyl alcohol, a mixture of five of the isomers of amyl alcohol) or any hydrocarbon solvent. Low-boiling mercaptans should be weighed in sealed ampoules (see Section 25). The contents of the flask is then titrated with the standard cupric alkyl phthalate solution. The copper solution is added in increments of about 0.5 ml until the endpoint is almost reached. The endpoint is the persistence of the blue-green color of the reagent. The solution may darken during the titration but it clears before the endpoint. The endpoint can be observed more readily when the flask is illuminated and viewed against a white background.

In some cases, particularly with mercaptans of low molecular weight, the yellow cuprous mercaptide precipitates, but in others it remains dissolved. In either case, the endpoint is readily discernible. A definite amount of copper reagent is necessary to impart a green color to 50 ml of solution. The amount is small but significant. A blank must therefore be run to determine the amount of reagent necessary to produce the color.

Calculations.

$$1 \text{ ml of } 1\ N \text{ cupric alkyl phthalate} = 0.0331 \text{ g of } -\text{SH}$$

If V = volume used in titration, N = normality of copper reagent, and W = weight sample,

$$\frac{(V\text{-blank}) \times N \times 0.0331 \times 100}{W} = \%\ -\text{SH}$$

This method is quite generally applicable to all types of mercaptans. Two mercaptans which cannot be determined are thioglycolic acid and dithioethylene glycol. Methyl and butyl thioglycolates, however, behave normally.

Mercaptans used to test this procedure were pentadecanethiol; methyl and butyl thioglycolate; terpene mercaptan; *n*-butyl, isobutyl, *tert*-butyl, lauryl and cetyl mercaptans; 2-mercaptoethanol.

This method is accurate and precise to about $\pm 1\%$.

Adapted from the Silver Method of Kolthoff and Harris

(*Ind. Eng. Chem.*, Anal. Ed., **18**, 161–162 [1946].)

$$AgNO_3 + RSH \rightarrow RSAg + HNO_3 \text{ (removed by } NH_4OH)$$

A rotating platinum wire electrode is the indicator in this method. The mercaptan is titrated with silver nitrate. When excess of silver ion is added, an increase in current is noted on a microammeter. A plot of current versus milliliters of reagent will show a sharp break at the endpoint (see Fig. 16).

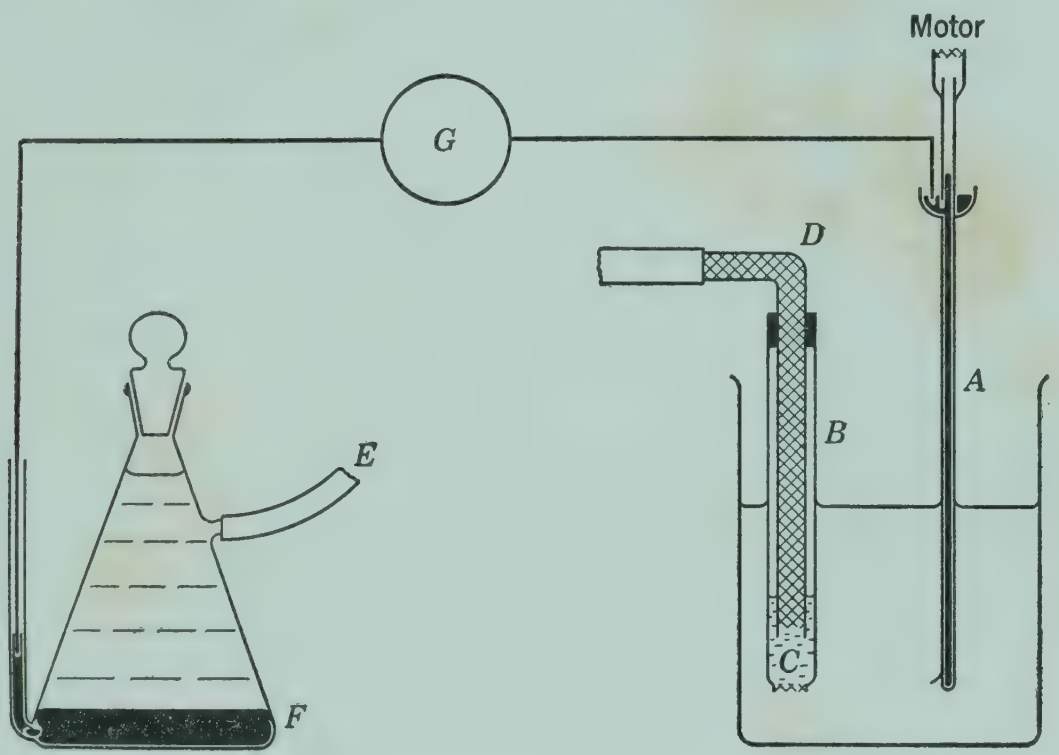


FIGURE 14. Amperometric titration apparatus.

If the titration is carried out in ammoniacal solution, chlorides and small amounts of bromides will not interfere.

Apparatus (Figs. 14 and 15.)

The platinum wire electrode (Fig. 15) is 6 to 8 mm long and 0.5 mm in diameter. An ordinary stirring motor can be used to rotate the electrode.

The reference electrode (*F*) has a potential of −0.23 volt (against the saturated calomel electrode). The electrolyte for the reference half cell is prepared by dissolving 4.2 g of potassium iodide and 1.3 g of mercuric iodide in 100 ml of saturated potassium chloride solution. A layer of mercury serves as the electrode. The salt bridge (*E*) consists of a rubber tube 2 ft in length and 6 mm inside diameter filled with saturated potassium chloride solution. The rubber tubing is connected to a short length of glass tubing (*D*), which is filled with a gel of 3% agar and 30% potassium chloride. To minimize contamination, a coarse sintered glass disk may be

inserted. For further protection against contamination with iodide, the tube (*B*), having an agar or fine sintered glass plug, can be inserted over the agar-filled glass tube (*D*). The electrolyte solution (*C*) (saturated potassium chloride) used in the tube (*B*) can be rinsed out and replaced as soon as it becomes contaminated with iodide. It is essential that all sources of high resistance such as air bubbles be eliminated from the salt bridge.

To complete the circuit, the two half cells are short circuited through the microammeter (*G*.) For the experiments used to test this procedure, a Weston (Weston Electrical Instrument Corporation, Newark, New Jersey), Model 430, microammeter was used. Instead of the microammeter, other current-indicating devices may be used such as a pointer galvanometer with a sensitivity of 0.25 μa per division on the attached scale.

FIGURE 15. Rotating platinum electrode.

Reagents.

0.005 *N* standard silver nitrate.
95% ethanol.
Concentrated ammonium hydroxide.
Ammonium nitrate.

Procedure. A sample containing about 5 mg of mercaptan sulfur is placed in a 250-ml beaker and dissolved in 100 ml of 95% ethanol. The solution is made about 0.25 *M* with respect to ammonium hydroxide and 0.01 to 0.1 *M* with ammonium nitrate. The end of the salt bridge and the rotating platinum electrode (*A*) are immersed in the solution, and the solution is titrated with 0.005 *M* silver nitrate. The diffusion current is read after each addition, and the increments of silver nitrate solution should be small when close to the endpoint. A few additional small portions of silver nitrate solution should be added when the ammeter shows that the endpoint has been passed. The ammeter readings are then plotted versus the milliliters of silver nitrate added. The curve will look as shown in Fig. 16.

A straight line is drawn through the points before the endpoint and another straight line through the points just after the endpoint. The intersection of these lines will correspond to the endpoint. There is usually no recordable current before the endpoint in these titrations.

The platinum electrode may become insensitive or erratic after

long use or when titrating large amounts of mercaptans. The sensitivity can be restored by wiping the electrode with a cloth.

Some samples affect the platinum electrode badly. As soon as the first excess of silver nitrate is indicated on the ammeter, the rotating electrode is stopped and wiped. Another small increment of silver nitrate is added, the rotating is started again, and the reading taken. This is repeated; the electrode is wiped after addition of each portion of silver nitrate and the reading is taken immediately after the electrode is started.

The electrodes should be thoroughly cleaned with concentrated nitric acid when it is deemed necessary. When a new or freshly

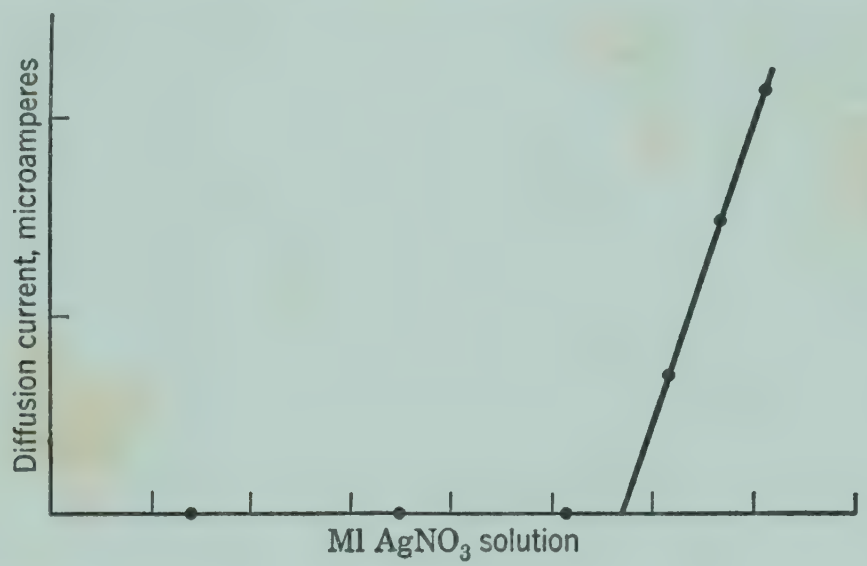


FIGURE 16.

cleaned electrode is placed in an ammoniacal silver solution and the cell is short-circuited, a large current of 20 to 30 microamperes may be observed. The current decreases rapidly and is practically zero after 5 to 10 minutes.

This procedure is very accurate and is simple to carry out. It might be well to mention here that this same type of amperometric titration can be used to determine halides, alone and in the presence of each other, and cyanides (see references quoted on p. 85). Samples containing more than 2 mg of mercaptan sulfur can be determined with the accuracy and precision of $\pm 0.3\%$. Substances used to test this procedure were ethyl, amyl, *n*-dodecyl, and cyclopentyl mercaptans, and also mercaptans in the C_{12} range whose purity was predetermined by another method.

General Reference

KLASON and CARLSON, *Ber.*, **39**, 738 (1906).

14 Dialkyl Sulfides (RSR)

This type of compound is readily oxidized according to the following reactions;

$$R_2S + (O) \rightarrow \underset{\text{Sulfoxide}}{R_2SO} \xrightarrow{(O)} \underset{\text{Sulfone}}{R_2SO_2}$$

The reaction to the sulfoxide goes very rapidly; the reaction to the sulfone is less rapid. Therefore, when oxidation is used as a quantitative means for determining alkyl disulfides, the results are high when determined by reaction to sulfoxide and low when determined by reaction to sulfone.

The method described below does away with the difficulties of the oxidation by titrating with the oxidizing reagent until an excess is noted. The oxidant is bromine, and the titration is carried out until a slight excess bromine is detected. In this way there is never a large excess of bromine present to carry the reaction beyond the sulfoxide. The bromine color endpoint does fade because of oxidation to sulfone, but it fades so slowly that the endpoint can be detected without difficulty. If the analysis is carried out by adding excess bromine and determining the excess bromine, the presence of the excess bromine accelerates the reaction to sulfone and high results are obtained.

This procedure, with slight modification (see pp. 95–97), may also be employed to determine alkyl disulfides. A method for determining alkyl sulfides and disulfides in the presence of each other is described on pp. 96–97.

Samples containing mercaptans can be analyzed provided they contain no more than 10% mercaptan. This procedure can be used to determine mercaptans with a precision of about ±0.8% but with an accuracy of only ±5% of the amount contained in the sample. This procedure, then, will determine both the sulfide and the mercaptan; the mercaptan alone can be determined by one of the methods described in the section on mercaptans. The sulfide content is then determined by difference. Since the accuracy of this method for mercaptans is only ±5%, if the sample contains too much mercaptan, a serious error in the sulfide value can be introduced when the mercaptan value is subtracted.

However, if the mercaptan content of the sample is less than 10% the absolute error which can be introduced into the sulfide value is small.

Adapted from the Method of Siggia and Edsberg

(*Ind. Eng. Chem.*, Anal. Ed., **20,** 938–939 [1948].)

Reagents.

0.1 *N* bromate-bromide (2.78 g of dry potassium bromate and 10 g of potassium bromide are dissolved in water and diluted to a liter). The solution is standardized by adding 6 ml of 6 *N* sulfuric acid to a 50-ml aliquot of solution in a 250-ml iodine flask. The flask is chilled in an ice bath, and 10 ml of 20% potassium iodide is added by placing the potassium iodide solution in the well and raising the stopper carefully so that the solution flows into the flask without allowing the bromine vapors to escape. The flask is shaken for 2 to 3 minutes to allow the bromine vapors to react with the potassium iodide. The iodine liberated is titrated with standard 0.1 *N* thiosulfate, starch indicator being used.

Glacial acetic acid.

Concentrated hydrochloric acid.

Procedure. A sample containing about 0.002 mole of alkyl sulfide is weighed into a 250-ml Erlenmeyer flask and dissolved in 40 ml of glacial acetic acid. To this solution is added 10 ml of water (less water can be added if the sample comes out of solution). Next 3 ml of concentrated hydrochloric acid is added and the solution is titrated with 0.1 *N* bromate-bromide solution until the first yellow color due to excess of bromine is detected. The endpoint is not so sharp as a starch-iodine endpoint but it is easily distinguished. A blank is run on the solvents alone to correct for the amount of excess bromine needed to detect the endpoint. The blank amounts to about 0.25 ml.

Calculations.

$$\frac{\text{Milliliters of } BrO_3^- - Br^- \times N \times \text{mol. wt. alkyl sulfide} \times 100}{\text{Grams sample} \times 2000} = \% \text{ alkyl sulfide}$$

The above procedure is accurate to better than 0.5% if large titrations (about 40 ml) are used. This minimizes endpoint errors; the endpoint is reproducible to about 0.1 ml.

Diethyl, di-*n*-butyl, diisobutyl, and dibenzyl sulfides were used to standardize the procedure.

15 Alkyl Disulfides (RSSR)

Alkyl disulfides are readily reduced to the corresponding mercaptans. The mercaptans can be determined by the procedures described in Section 13. Disulfide samples containing mercaptans can be determined by first analyzing the unreduced sample for mercaptan and then reducing the disulfide and determining the total mercaptan; the disulfide content is obtained by difference. When pure disulfides are analyzed, the procedure described below yields results about 4% low. However, the lower the percentage of disulfide in the sample, the more complete will be the reduction and the closer the analysis will be to the true amount of disulfide.

The reaction between bromine and disulfides provides another method for disulfide determination. This method has a higher degree of accuracy and reproducibility than the reduction method but it has the disadvantage of being affected by a larger number of interfering compounds.

REDUCTION METHOD

Adapted from the Method of Kolthoff, May, Morgan, Laitinen, and O'Brien

(*Ind. Eng. Chem.*, Anal. Ed., **18,** 442–444 [1946].)

This method employs a Jones reductor to reduce the alkyl disulfides.

Reagents.

0.3 *N* sulfuric acid in 90% ethanol.

Zinc amalgam (0.02% in mercury): By stirring with 6 *N* hydrochloric acid for one minute 100 g of 20-mesh granulated zinc is etched. Next, 27 mg of mercuric chloride is added, and, after being stirred for one minute, the zinc is washed with distilled water. The Jones reductor should be of convenient size to hold 100 g of 20-mesh zinc.

10 *N* sulfuric acid: The reagents for the determination of the mercaptan formed by the reduction are given with the procedures for mercaptans (Section 13).

Procedure (Fig. 17). A sample containing about 0.0003 mole of disulfide is dissolved in 90% ethanol that is 0.3 *N* in sulfuric acid, and

25-ml portions of the solution are heated to 50°C and are allowed to flow through the reductor, which is immersed in a bath held at 50°C. The rate of flow is such that the disulfide solution remains in contact with the amalgamated zinc for about 5 minutes. The reductor is then rinsed quantitatively with three successive 25-ml portions of 0.3 *N* sulfuric acid in 90% ethanol. The solution is analyzed for mercaptan. The authors of this paper used the amperometric silver method described on pp. 89–91; however, any of the other procedures described in Section 13, except possibly the iodimetric method, may be used.

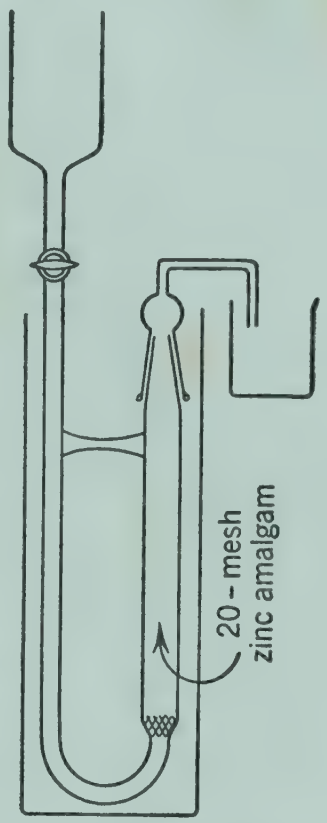


FIGURE 17. Disulfide reduction apparatus.

Incomplete reduction occurs if the reductor is not completely clean. For cleaning, a 10 *N* sulfuric acid solution is used. The reductor should be checked with a standard disulfide solution before each series of runs to be sure that it is in working order. If the reductor remains slow even after rinsing with 10 *N* sulfuric acid, it should be refilled with fresh zinc amalgam.

If the sample contains mercaptan, the mercaptan is determined on an unreduced sample. A sample is then reduced, and the total mercaptan is determined. The difference will yield the disulfide content. This method was tested only with *n*-dodecyl disulfide.

BROMINATION METHOD

The bromination procedure as described for alkyl sulfides (pp. 92–93) can also be used to determine disulfides. This method is more accurate, precise, and simple than the reduction method described, but more compounds interfere.

The reaction is:

$$RSSR + 5Br_2 + 4H_2O \rightarrow 2RSO_2Br + 8HBr$$

For most disulfides more acid is required to achieve complete reaction than is needed for alkyl sulfides. The alkyl sulfides require only 3 ml of concentrated hydrochloric acid. Table 4

TABLE 4

Sample	*Milliliters of Acid Used*	*Percentage Found*
Butyl disulfide in 50 ml of 80% glacial acetic acid and 20% water	3 ml conc. HCl	87.2
	5 ml conc. HCl	92.2
	10 ml conc. HCl	95.8
	25 ml conc. HCl	96.8
	5 ml 6N H_2SO_4	79.6
	5 ml 12N H_2SO_4	83.0
	10 ml 12N H_2SO_4	86.9
	10 ml conc. H_2SO_4	97.8
	20 ml conc. H_2SO_4	98.8
	25 ml conc. H_2SO_4	98.8
	30 ml conc. H_2SO_4	98.8
Ethyl disulfide in 50 ml of 80% glacial acetic acid and 20% water	3 ml conc. HCl	71.3
	10 ml conc. HCl	86.4
	20 ml conc. H_2SO_4	95.6
	25 ml conc. H_2SO_4	95.3
	30 ml conc. H_2SO_4	95.3
Phenyl disulfide in 50 ml of 80% glacial acetic acid 20% water	3 ml conc. HCl	94.5
		95.0
		94.3
	25 ml conc. HCl	94.0
l-Cystine in 50 ml of water	3 ml conc. HCl	100.0
		99.6
	25 ml conc. HCl	99.6
	20 ml conc. H_2SO_4	99.6

shows the amounts of acid needed for various disulfides. When in doubt as to the amount of acid to use for a particular disulfide it is best to use 25 ml of concentrated sulfuric acid, since this amount of acid is great enough to work for the most inert disulfides. Disulfides that will react quantitatively with much less acid will also react satisfactorily in the presence of 25 ml of concentrated sulfuric acid. Also, it is best to titrate the solution while it is warm (about 30° to 50°C). Solutions which are cooled below these temperatures react too slowly to give a sharp endpoint.

Mixtures of alkyl sulfides and alkyl disulfides can be determined by first obtaining the total of the two types by the bromination method just discussed and then determining the disulfide by the reduction method described above. The difference between the

two analyses will yield the amount of the alkyl sulfide. The accuracy and precision of this technique are limited by the reduction method for disulfides, the accuracy and precision of which are rather low (see p. 94).

Samples containing mercaptans can also be determined, provided the conditions are the same as those described for alkyl sulfides (p. 92).

The following were used to test this procedure: ethyl, *n*-butyl, phenyl disulfides, and *l*-cystine.

Calculations.

$$\frac{\text{Milliliters of } BrO_3^- - Br^- \times N \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 10{,}000} = \% \text{ compound}$$

16 Sulfonic Acids and Salts

SULFONIC ACIDS

Free sulfonic acids, which have strengths comparable to sulfuric acid, can be titrated with little difficulty by using standard sodium hydroxide. If the sample is insoluble in water, it can be titrated in alcohol with alcoholic sodium hydroxide, or the sample can be dissolved in excess aqueous sodium hydroxide and the excess hydroxide can be titrated. Phenolphthalein can be used as the indicator.

SULFONIC ACID SALTS

IGNITION

Salts of sulfonic acids are very difficult to determine. They are rather unreactive and are so nearly neutral that they cannot be titrated as can some carboxylic acid salts. The only successful method is ignition of the sample in the presence of some sulfuric acid to yield the sulfate of the cation of the salt. The sulfate is then weighed. The method is accurate but is applicable only to those samples which contain no cation other than that attached to the sulfonic acid.

Procedure. About 2 g of sample is weighed into a tared crucible (platinum is preferable but porcelain can be used). The sample is just covered with concentrated sulfuric acid. The crucible is heated with a Bunsen burner, care being taken not to heat too vigorously and cause spattering of the contents of the crucible. The heating is continued until fumes of sulfur trioxide are no longer visible. The crucible is then strongly ignited in a muffle furnace (a Meker type burner can be used) until the contents are white. The ignition residue is cooled and moistened well with sulfuric acid, and the heating with a Bunsen burner is repeated until all the acid has been driven off. The crucible is then ignited in the muffle oven for one-half hour, cooled, and weighed.

Calculations.

$$\frac{\text{Grams residue} \times \text{gravimetric factor} \times 100}{\text{Grams sample}} = \% \text{ salt}$$

The procedure is applicable only when the sample contains no cation other than that of the salt. If such cations are present they too would form sulfates and be weighed in the residue. This is the best method for the determination of sulfonic acid salts, but there is often some inorganic material present which makes this method useless.

PRECIPITATION

There is another method which has sometimes been found useful. Many sulfonic salts will be precipitated with such cations as barium, silver, or mercury (-ous or -ic). Barium is the most general precipitant, but sometimes only silver or mercury ions will work. A pure sample of the salt to be determined should first be tested to see if it precipitates quantitatively with any of the cations mentioned above. When a convenient cation is found, it must be ascertained whether there are any materials in the actual samples which might interfere; for instance sulfate ions in the sample would exclude the use of barium, and chloride ions would exclude silver and mercurous ions. If these conditions can be met, the procedure is applicable. It must be remembered that if carboxylic groups are present, they also may react with a metallic ion.

This method is of such a nature that a general procedure cannot be described. Each sample has to be treated in a special way. In most cases, after precipitation is carried out, the precipitate is filtered through a tared Gooch or sintered-glass crucible, and then is washed, dried, and weighed. If a precipitated barium salt is waxy, hygroscopic, or in any other way difficult to determine accurately, the precipitate can be soaked with concentrated sulfuric acid and ignited to the sulfate as described in the above ignition procedure. This ignition will not work well for silver or mercury precipitates. Free sulfonic acid in a salt sample has to be determined on a separate sample, and the amount present has to be subtracted from the total acid plus salt as determined by precipitation.

I 7 Peroxides

Peroxides are rather easily determined because of their oxidative properties. Peroxides will oxidize iodide ion to free iodine and will oxidize arsenite ion to arsenate; both these reactions can be used to determine peroxides. The liberation of iodine from potassium iodide is the most rapid method, but it can be used only in two common organic solvents and cannot generally be used in the presence of unsaturated compounds. The arsenious oxide method can be applied in many common organic solvents; also unsaturated compounds do not interfere.

IODOMETRIC DETERMINATION OF PEROXIDES

The procedure described below involves the liberation of iodine from potassium iodide in an organic solvent. There is some effect of the solvent on the reaction since in some solvents the reaction does not proceed quantitatively. The best solvents for the reaction are ethanol and isopropanol. Glacial acetic acid can be used, but a blank is necessary on this solvent.

Adapted from The Method of Kokatnur and Jelling

(*J. Am. Chem. Soc.*, **63,** 1432–1433 [1941].)

Reagents.

C.p. absolute ethanol or isopropanol.
0.1 *N* standard sodium thiosulfate.
Saturated aqueous potassium iodide solution.

Procedure. A sample containing about 0.001 to 0.002 mole of active oxygen is weighed into a 250-ml iodine flask and is dissolved in 25 to 50 ml of the alcohol being used. One ml of saturated potassium iodide solution and 1 ml of glacial acetic acid are added and the flask is stoppered with the ground-glass stopper which has been wet with solvent. The solution is heated to almost boiling and is kept at incipient boiling for 2 to 5 minutes with occasional swirling. Water slows the reaction between the peroxide and the

potassium iodide. If samples contain considerable amounts of water, they may have to be heated for a longer time in order to complete the reaction. Without cooling, the solution is titrated with 0.1 *N* thiosulfate to the disappearance of the yellow iodine color. Starch cannot be used as an indicator in alcohol. The reaction between iodine and thiosulfate is instantaneous and the endpoint is easily discernible. For samples with small amounts of peroxide, 0.05 *N* thiosulfate can be used and the endpoint is still readily detected.

No blank is needed in this method. Allowing a blank solution to stand for 24 hours in an open flask (not in direct sunlight) results in only a very slight iodine coloration.

Calculations.

$$\frac{\text{Milliliters thiosulfate} \times N \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 2000} = \% \text{ peroxide compound}$$

The above procedure is reproducible to ±0.5%. Low results may be obtained by boiling the solution instead of keeping the flask at incipient boiling. Methanol and acetone are unsuitable as solvents. Methanol reacts with the reagents while acetone gives low and inconsistent results.

Benzoyl peroxide and hydrogen peroxide were used to test the procedure. These were the only peroxides which could easily be obtained pure, or whose purity is easily ascertained.

DETERMINATION OF PEROXIDES USING ARSENIOUS OXIDE

Need often arises for the determination of peroxides in media in which methods involving the liberation of iodine from potassium iodide cannot be used. In the discussion of the above procedure it was shown that the only common organic solvents in which the iodometric methods will work efficiently are 2-propanol and ethyl alcohol. In the case of peroxides in unsaturated organic compounds, such as may occur in polymerization work, the iodometric methods present the difficulties of addition and oxidation and cannot be used for these reasons.

In this method, arsenious oxide is used to reduce the peroxide. This method works in all the common organic solvents and also can be used to determine peroxides in monomers and polymers.

Reichert and coworkers[1] used arsenious oxide to determine various inorganic peroxides.

Procedure of Siggia

(*Ind. Eng. Chem.*, Anal. Ed., **19**, 827 [1947].)

Reagents.

0.1 *N* arsenious oxide standard solution containing 25 g of sodium bicarbonate per liter of solution.
1 *N* sulfuric acid.
Sodium bicarbonate.
0.1 *N* standard iodine solution.

Procedure. In a 125-ml Erlenmeyer flask, 25 ml of a standard 0.1 *N* arsenious oxide solution is introduced, containing 25 g of sodium bicarbonate per liter. To this is added a sufficient amount of peroxide-containing solution to yield about 0.005 to 0.010 g of active oxygen. Where the sample solutions are immiscible with water, ethanol is added until a homogeneous solution is obtained. When the sample is a polymer, it is dissolved in benzene, and the ethanol is added as before. Precipitation of the polymer does not affect results noticeably. However, if the peroxide content in the polymer is greater than 5% of benzoyl peroxide, it is best to pour off the supernatant liquid from the precipitated polymer, redissolve the polymer, and reprecipitate it with ethanol. Then the extracts are combined.

Boiling chips are added, and the solution is concentrated to about 25 ml with a stream of air flowing over the liquid to increase the rate of evaporation. Water is added until the volume is about 40 ml. This procedure is continued until practically all the monomer, alcohol, or other organic solvents are replaced with water. The solution is made barely acid with 1 *N* sulfuric acid, and then 0.5 g of sodium bicarbonate is added. The solution is chilled and the excess arsenious oxide is titrated with standard 0.05 to 0.1 *N* iodine to the appearance of the yellow iodine color. Large amounts of organic solvent in the water cause the final endpoint to develop very slowly. The endpoint is very sharp if most of the organic solvents are removed.

This method was sufficiently sensitive so that 0.008 g of active oxygen could be accurately determined to within ±0.5%. Hydro-

[1] J. S. Reichert, S. A. McNeight, and H. W. Rudel, *Ind. Eng. Chem.*, Anal. Ed., **11**, 194–197 (1939).

gen peroxide was also used together with the benzoyl peroxide to test the procedure.

No interference was noted when iodine was added to the excess arsenious oxide in samples that contained unsaturated compounds because the arsenious oxide reacts instantaneously with iodine.

TABLE 5

Results	*Grams of Active Oxygen*	
	Observed	*Calculated*
Benzoyl peroxide in CH_3OH	0.00387	0.00393
	0.00396	
Benzoyl peroxide in acetone	0.01304	0.01320
	0.01315	
	0.01303	
Benzoyl peroxide in benzene	0.00400	0.00394
	0.00397	
Benzoyl peroxide in styrene	0.00582	0.00581
	0.00581	
	0.00584	
Benzoyl peroxide in isobutyl vinyl ether	0.01322	0.01323
	0.01308	
Benzoyl peroxide in methyl methacrylate	0.01332	0.01323
	0.01335	
Benzoyl peroxide in polystyrene	0.00579	0.00581
	0.00581	

The benzoyl peroxide samples used above were recrystallized from acetone.

In addition, repeated boiling with water removed most of the organic material that might have interfered by reaction with iodine. Both these factors contribute to the lack of interference.

In the case of peroxides in polymers, it was found that not much peroxide was trapped in the polymer upon precipitation with ethanol. Benzoyl peroxide in polymers, in amounts less than 3%, could be determined with no noticeable loss of peroxide due to occlusion during the precipitation. In polymer samples containing more than 5% benzoyl peroxide, it was found best, after precipitation of the polymer with the ethanol, to pour off the supernatant liquid, redissolve the polymer in benzene, and reprecipitate it with ethanol, and then to combine the two liquid portions before analysis. This achieves a more complete extraction of the peroxide.

18 Isocyanates (RN=C=O) and Isothiocyanates (RN=C=S)

Both these compounds react readily with primary amines according to these equations:

$$RN{=}C{=}O + R_1NH_2 \rightarrow RNHC(=O)NHR_1$$

$$RN{=}C{=}S + R_1NH_2 \rightarrow RNHC(=S)NHR_1$$

The basis for the analysis described below is the addition of an excess of 1-*n*-butylamine to the sample followed by acidimetric titration of the excess amine.

The reaction is rapid at room temperature, and the procedure was found to be accurate and precise to ±0.5%.

Adapted from the Method of Siggia and Hanna

(*Ind. Eng. Chem.*, Anal Ed., **20.**, 1084 [1948].)

Reagents.

n-Butylamine 12.5 g, is diluted to 100 ml with purified dioxane. The dioxane is purified by allowing it to stand over sodium hydroxide pellets and by changing the pellets each day until they no longer become brown. The dioxane is then distilled from and stored over sodium hydroxide.

0.1 *N* standard sulfuric acid.

Phenolphthalein indicator.

Procedure. To a 100- or 150-ml glass-stoppered Erlenmeyer flask, 20 ml of *n*-butylamine reagent is charged. To the reagent is added the sample which should contain about 0.002 mole of the isocyanate or isothiocyanate. The flask is stoppered and is allowed to stand at room temperature for 15 minutes. A very few samples may require heating for a few minutes to complete the addition of the *n*-butylamine to the isocyanate or isothiocyanate. All heating should be done under reflux to prevent loss of *n*-butylamine. The condenser is rinsed with water. The con-

tents of the flask is then titrated with 0.1 *N* sulfuric acid, phenolphthalein being used as an indicator.

A blank should be run on 20 ml of the reagent in order to determine its strength.

Calculations.

$$\text{Milliliters of acid for blank} - \text{milliliters acid for sample} = A$$

$$\frac{A \times N\ H_2SO_4 \times \text{mol. wt. compound} \times 100}{\text{Grams sample} \times 1000} = \%\ \text{compound}$$

The following were used to test the above procedure: phenyl and 1-naphthyl isocyanates and methyl, ethyl, and phenyl isothiocyanates.

Interferences in this procedure consist of any acidic or basic impurities which may be included in the sample. These impurities should be determined by titration before the analysis for the isocyanate or isothiocyanate is made; the final analysis should then be corrected for these impurities.

Thiocyanates will interfere, since these compounds will isomerize to the isothiocyanate on heating.

19 Vinyl Ethers ($ROCH{=}CH_2$)

There are several methods for determining the vinyl ether grouping. One method has already been discussed (pp. 60–63); it consists of hydrolysis in the presence of acids to acetaldehyde, and the determination of the acetaldehyde by means of bisulfite addition.

$$ROCH{=}CH_2 + H_2O \xrightarrow{H^+} ROH + CH_3CHO$$

Another method consists of acid hydrolysis and determination of the aldehyde by a hydroxylamine method similar to the one on pp. 63–64. The method described below is best in that it is the simplest to carry out and is not affected by the impurities usually associated with vinyl ethers, such as free acetaldehyde, acetals, alcohols, acetylene, and water. The methods involving hydrolysis will be interfered with by acetals since acetals also hydrolyze in acid solution to yield aldehydes. Also, any free aldehyde will be measured by these hydrolysis methods.

The method described below involves reaction of the vinyl ethers with iodine and alcohol to form the corresponding iodoacetal (see equation below). The iodine consumed is a measure of the amount of vinyl ether present. The method has an average accuracy and precision of $\pm 0.2\%$ with a maximum deviation of 0.5%.

Adapted from the Method of Siggia and Edsberg

(*Ind. Eng. Chem.*, Anal. Ed., **20,** 762–763 [1948].)

$$ROCH{=}CH_2 + I_2 + CH_3OH \rightarrow ROCHCH_2I + HI$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad |$$
$$\qquad\qquad\qquad\qquad\qquad\qquad\qquad\quad OCH_3$$

Reagents.

0.1 *N* standard iodine solution (aqueous).
0.1 *N* standard sodium thiosulfate solution.
C.p. methanol.

Procedure. Into a 500-ml wide-mouth glass-stoppered bottle, 50 ml of methanol is introduced. The sample, sealed in a glass

ampoule (see Section 25) and containing about 0.001 mole of vinyl ether, is introduced into the bottle together with about 20 ml of glass beads to aid in breaking the ampoule.

Next, 50 ml of 0.1 *N* standard iodine is pipetted into the bottle. The bottle is then stoppered, and the glass stopper is greased to prevent leaks, especially when low-boiling ethers such as methyl vinyl ether are determined. The bottle is shaken to break the ampoule and is then allowed to stand for 10 minutes with occasional shaking. If the ether is very volatile, the bottle should be shaken for the full 10 minutes to insure reaction of the ether vapor in the atmosphere of the bottle. After the 10 minutes, the bottle is opened and the excess iodine is titrated with 0.1 *N* thiosulfate solution to the disappearance of the iodine color.

The very sharp endpoint can be obtained within one drop of thiosulfate. The iodine color will return on standing but does this so slowly that it will not interfere with the endpoint. If the sample is so large as to consume almost all the iodine, the iodine color at the endpoint will return more rapidly; in these cases the returning iodine color may interfere with the endpoint. However, if the size of the sample is chosen so as to use approximately one-half the amount of iodine introduced, there is no difficulty at the endpoint.

Calculations.

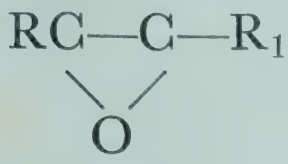
Milliliters of 0.1 *N* thiosulfate needed to titrate 50 ml of the iodine standard solution − milliliters of thiosulfate used to titrate sample = *A*

$$\frac{A \times N \text{ thiosulfate} \times \text{mol. wt. ether} \times 100}{\text{Grams sample} \times 2000} = \% \text{ vinyl ether}$$

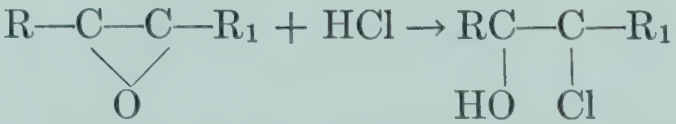
The above procedure was tested and was found to work well with the following alkyl vinyl ethers: methyl, ethyl, propyl, isopropyl, butyl, isobutyl.

Acetaldehyde, acetals, and acetylene were introduced into ether samples and were found not to interfere with the procedure.

20 Oxirane Oxygen (Epoxide Compounds)



The following analytical method is based on the reaction between epoxide compounds and hydrochloric acid to form the corresponding chlorohydrin.



The method[1] consists of the addition of a known excess of hydrochloric acid and the determination of the excess by titration with standard base. The reaction is rapid, and the accuracy and precision attainable are of the order of $\pm 0.5\%$.

Reagents.

Hydrochloric acid and calcium chloride solution: Eight hundred and ten grams of $CaCl_2 \cdot 2H_2O$ is dissolved in 600 ml of water. To this is added 95 ml of concentrated hydrochloric acid.

1 *N* Standard sodium hydroxide.

Phenolphthalein indicator.

Procedure. In a 500-ml wide-mouth, glass-stoppered bottle 100 ml of the hydrochloric acid and calcium chloride solution is placed. The sample, containing 0.04 to 0.06 mole of oxirane oxygen, is sealed in a glass ampoule (see Section 25) and placed in the bottle with the reagent. Some glass beads are added to the bottle to facilitate the breaking of the ampoule. The bottle is then stoppered, and the stopper is greased to prevent leakage. The bottle is shaken vigorously for 3 minutes to break the ampoule. With low-boiling epoxide compounds such as ethylene oxide, the bottle and contents should be cooled before the ampoule is broken.

[1] The method is essentially that of F. W. Kerchov, *Z. anal. Chem.*, **108**, 249 (1937), with some modification toward simplification and toward an increase in precision.

For cooling, the bottle should be stoppered as before and placed up to its neck in ice water until crystals of calcium chloride begin to separate out.

After it is shaken, the bottle is opened and 200 ml of water is added to rinse the sides of the bottle and the stopper. The contents are then titrated with 1 N sodium hydroxide to the phenolphthalein endpoint.

Calculations.

A = milliliters 1 N NaOH for 100 ml of HCl – $CaCl_2$ reagent alone

B = milliliters 1 N NaOH for sample

$$\frac{(A - B) \times N \text{ NaOH} \times \text{mol. wt. epoxide compound} \times 100}{\text{Grams sample} \times 1000} = \% \text{ epoxide compound}$$

Ethylene oxide, propylene oxide, and butadiene monoxide were used to test the above procedure. All samples were purified and distilled before use.

21 Water in Organic Compounds

It is often necessary to determine the water which may be found in samples of organic compounds. This determination can readily be carried out by the method of Mitchell, Hawkins, and Smith. Karl Fischer reagent as described on pp. 67–68 being used. It must be remembered that a blank must be run on the solvent to correct for the water contained therein. Solvents with too high a water content should not be used to dissolve the sample, since this would yield a large blank and the analysis would then depend on the difference between two large figures, which usually leads to a decreased accuracy. Methanol and pyridine serve well as solvents.

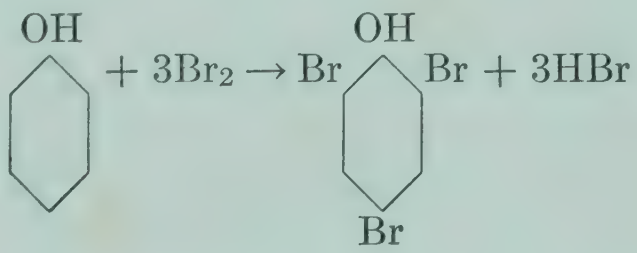
The procedure is widely applicable; interfering compounds are those which react with iodine. When very high results for water are obtained, a dry sample should be tested with the Karl Fischer reagent to make sure that the high results are not caused by reaction of the reagent with the organic material in which the water is dissolved.

22 Miscellaneous Procedures

BROMINATION PROCEDURE—PHENOLS, AMINES, SOME HYDRAZINES

Bromination is an indirect method for determining hydroxyl groups on a benzene ring. Benzene rings which contain a phenolic hydroxyl group will substitute bromines readily for the hydrogens in the ortho and para positions. Phenol itself will substitute bromine in all three positions to form 2,4,6-tribromophenol. When one of these positions is occupied, the bromine will substitute on the unoccupied ortho or para positions.

The use of aqueous bromine is not feasible since the solution is unstable. A solution of potassium bromate and potassium bromide is used instead. When acid is added to this solution, bromine is liberated. The excess bromine is determined iodometrically.



Method from Scott's Standard Methods of Chemical Analysis

(Adapted with permission from Wilfred Scott, *Standard Methods of Chemical Analysis*, 5th ed., edited by N. Howell Furman, Vol. II, p. 2253, D. Van Nostrand Co., New York, 1939.)

Reagents.

0.1 *N* bromate-bromide: 2.78 g of anhydrous, c.p. potassium bromate, and 10 g of potassium bromide are dissolved in water and diluted to one liter.

Procedure. A sample containing 0.02 equivalent of compound is dissolved in water and diluted to a liter. An insoluble phenol is dissolved in a minimum of aqueous sodium hydroxide before dilution; an insoluble amine is dissolved with the aid of a few

milliliters of concentrated hydrochloric acid. A 100-ml aliquot is taken in a glass-stoppered iodine flask, and 50 ml of bromate-bromide reagent is added. The flask is shaken to mix the reactants and then 5 ml of concentrated hydrochloric acid is added. The solution is shaken again and is allowed to stand 15 minutes; then 2 g of potassium iodide is added, and the liberated iodine is titrated with 0.1 N thiosulfate.

A blank run is made on 50 ml of the bromate-bromide solution.

$$\text{Milliliters for blank} - \text{milliliters for sample} = A$$

$$\frac{A \times N \text{ thiosulfate} \times \text{mol. wt. of} \begin{Bmatrix}\text{amine}\\\text{phenol}\end{Bmatrix} \times 100}{\text{Grams sample} \times 2000 \times B} = \% \begin{Bmatrix}\text{amine}\\\text{phenol}\end{Bmatrix}$$

B = number of bromine atoms that will substitute on the particular phenol or amine being determined

Calculations for hydrazines are as shown on p. 75 for the iodine method.

The procedure as described in Scott's *Standard Methods of Chemical Analysis* was intended only for phenol. However, the procedure was found to work very well for other phenols and also for amines. Materials which have been determined successfully are phenol; resorcinol; *p-tert*-amyl-, *p-tert*-butyl-, *p-n*-octyl-, and *o-n*-octylphenol and also aniline; N-ethylaniline; and hydrazine sulfate.

Alkylated phenols or anilines should not be kept in contact with the bromine for more than 3 minutes since bromine will tend to substitute on the alkyl groups and make the results slightly higher. Unsaturated compounds will of course interfere, since they add bromine. Some water-soluble unsaturated compounds may be determined by this method, but the method is not so general for unsaturated compounds as the Lucas and Pressman method on pp. 33–37.

A few phenols contain hydroxyl groups whose hydrogens are acidic enough to be titrated with sodium hydroxide.

Although aromatic hydrazines react with bromine they cannot be determined by this method. The reaction involved is the oxidation of the hydrazine to the corresponding diazonium compound.

Most aliphatic hydrazines react quantitatively and can be determined by this method. Aromatic hydrazines, however, react very slowly.

HYDROGENATION PROCEDURE TO REMOVE INTERFERENCE OF ALIPHATIC UNSATURATED CARBON-CARBON COMPOUNDS PRESENT IN SAMPLE TO BE BROMINATED

Very often, samples to be determined by bromination contain some unsaturated material which interferes by addition of bromine, causing high results. This interference can be removed by hydrogenation of the sample. Hydrogenation can also be employed to remove the interference of alkyl vinyl ethers in acetal analyses (see pp. 59–64). Two catalysts can be used—Adams catalyst (platinum oxide) and Raney nickel. Adams catalyst is more active, and in certain cases it will saturate benzene rings. This is unsatisfactory because the resultant cyclohexanes often are very reactive toward bromine.

Apparatus.

(See Fig. 18.)

Procedure. A sample is weighed into the reaction flask *F* and is dissolved in a convenient inert solvent. The catalyst is then added, about 0.5 g of catalyst per 20 ml of solution being used. (The Adams catalyst can be recovered and reused. Because of the reactivity of Raney nickel with atmospheric oxygen, it is best to discard this catalyst after each determination.) A glass-encased iron paddle is put in the flask; this paddle is used to stir the solution by using a rotating magnet outside the flask. Condenser *D* is attached to the flask; the condenser is attached to the manifold *C* and stopcock *H* is opened. All joints should be greased to prevent leakage. The reaction flask is chilled in ice water. If the sample is volatile, a mixture of dry ice and methanol should be used to chill the flask. Stopcock *E* is opened, and the apparatus is flushed for 5 minutes with a slow stream of hydrogen (one bubble per minute when a bubble tower is used to control the flow rate of the hydrogen). Stopcock *E* is then closed, and the cooling bath is removed from around the reaction flask. The mercury level in the manometer *B* is forced down by the

increasing hydrogen pressure. The hydrogen flow can be controlled by a reducing valve on the hydrogen tank so that a constant pressure (should not exceed 30 cm or the ground-glass joints will be forced open) can be maintained without any excess hydrogen bubbling out the open end of the manometer. The escape of hydrogen through the manometer does not hurt the sample but it does increase the explosion hazard. The mixture of sample solution and catalyst is stirred by rotating a magnet outside the flask. Commercial magnetic stirrers can be purchased from the

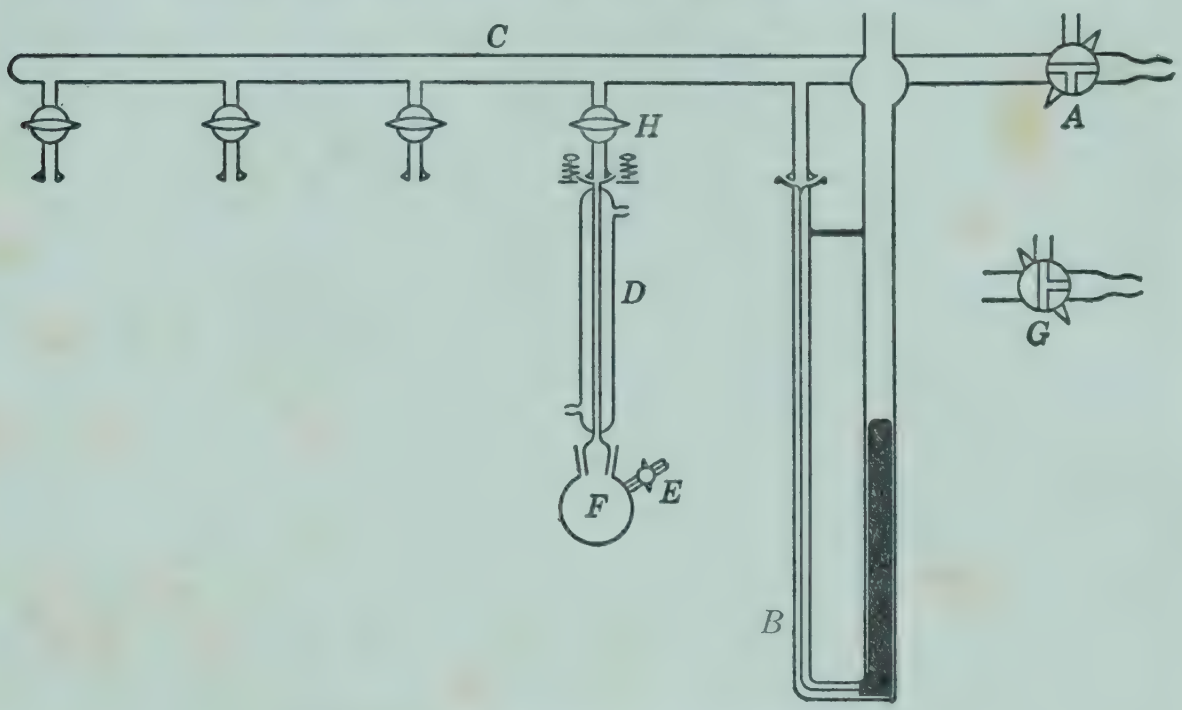


FIGURE 18. Hydrogenation apparatus.

A. H. Thomas Company. However, a magnetic stirrer can be made by affixing a permanent magnet to a metal shaft. The magnet can then be attached to a stirring motor and rotated under the flask to rotate the iron paddle in the flask.

To test for complete hydrogenation, stopcock *A* is turned to position *G* and the level of the mercury in the capillary *B* of the manometer is observed. If the mercury level rises on continued stirring of the sample, the sample is still consuming hydrogen. Stopcock *A* is turned back to the original position, and the hydrogenation is continued. The hydrogenation is continued until the mercury level in the capillary *B* no longer rises when stopcock *A* is in position *G* and the sample is being stirred. (Stirring should be continued during the test for complete hydrogenation because hydrogen is consumed fastest when the sample is stirred. An

unstirred sample may not consume any hydrogen, while still containing some unsaturation.)

After completion of hydrogenation, the catalyst is removed from the sample by centrifuging or by filtration. The precautions noted on pp. 39–40 are observed. The sample can then be brominated without any interference.

The time required for hydrogenation varies with the size of the sample, the amount of catalyst used, the hydrogen pressure, and the rate of stirring. Hydrogen pressure of 30 cm of mercury is convenient to achieve hydrogenation without forcing open the ground-glass joints. Stirring should be as vigorous as possible. The amount of catalyst used is best determined by the volume of the solution being hydrogenated, since the "concentration" of catalyst affects the hydrogenation rate (about 0.5 g of catalyst per 10 ml of solution is a convenient catalyst "concentration").

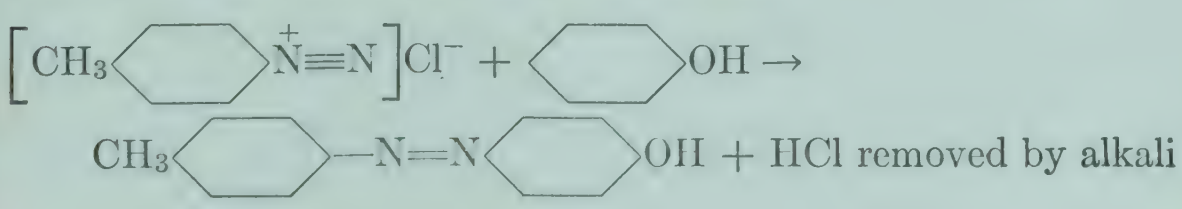
The apparatus is also capable of hydrogenating four samples simultaneously. If only one hydrogenation is being carried out, the other three stopcocks on the manifold are kept closed. If more than one hydrogenation is being run, each reaction flask is flushed with hydrogen individually, starting with the reaction flask nearest the manometer. To test any of the reactions for completeness, the manifold stopcocks leading to the other reaction flasks are closed while the particular reaction is being tested. Raney nickel can cause fires if it is filtered from the sample after hydrogenation and allowed to dry on the filter. Raney nickel is usually stored under a higher boiling alcohol such as propyl or butyl.

CAUTION

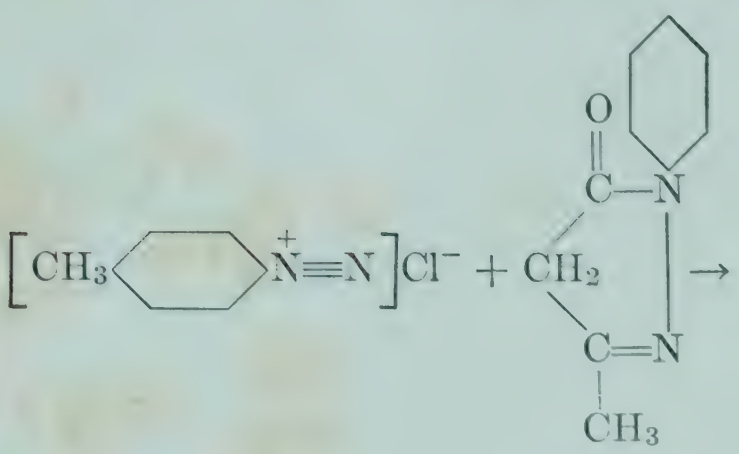
See pp. 39–40.

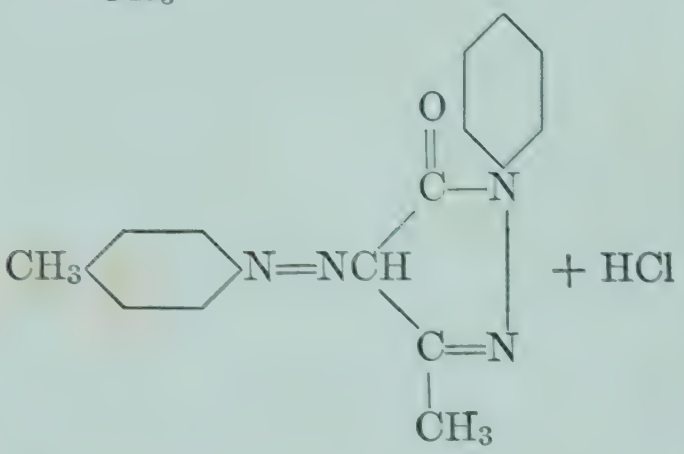
COUPLING: PHENOLS, ACTIVE METHYLENE COMPOUNDS, SOME AMINES, AND DIAZONIUM COMPOUNDS

Phenolic compounds will couple readily with diazonium compounds in alkaline solution. Compounds with active methylene groups will also couple as will some amines.



The coupling takes place in the para position on the phenol or amine unless this position is occupied, in which case the coupling may take place on the ortho position, though the reaction at the ortho position will usually be considerably slower. Some phenols and amines will couple first in the para position and then will commence to couple in the ortho position. The compounds containing active methylene groups will couple at these groups.





The coupling procedures vary somewhat, depending on the reactivity of the compounds involved. Some compounds will couple in a solution at pH = 5; others will couple only in very alkaline solution. The faster the coupling the better the analysis, since the diazonium compound is unstable. The more alkaline the solution the faster the coupling. However, the more alkaline the solution the faster the diazonium salt will decompose. In general, salts like sodium acetate and sodium bicarbonate will be sufficiently alkaline for most couplings. Sodium carbonate is sometimes used, while sodium hydroxide is seldom resorted to, since it decomposes the diazonium salt too readily.

The coupling procedures are, on the average, accurate to about $\pm 1\%$. These methods are not so accurate as we should like, but

they are definitely useful in the determination of samples whose interfering action makes other methods of analysis impossible. The acetylation methods for amines and phenols described on pp. 4–7 are more accurate, however, if a sample contains some alcohol or other compounds which will acetylate, these methods are of no use. It is in cases like these that couplings can be used. Analytical coupling procedures are very generally used in industrial analytical laboratories.

The following procedure is a very general coupling method which can be readily modified to deal with samples requiring different treatment.

Reagents.

Two diazonium salts can be used as standards for coupling. These are the *p*-toluene- and *m*-nitrobenzene-diazonium chlorides.

0.1 *N* *p*-toluene diazonium chloride standard solution: First, 100 ml of 0.5 *N* *p*-toluidine stock solution (53 g of *p*-toluidine plus 131 ml of concentrated hydrochloric acid diluted to a liter with water) is introduced into a 500-ml volumetric flask, and the temperature of the flask is reduced to 15 to 20°C. Next, 50 ml of 1 *N* sodium nitrite (temperature 15 to 20°C) is added, and the resultant solution is diluted to 500 ml. The solution is shaken to assure thorough mixing. A slight excess of nitrous acid should be indicated on starch-iodide paper. The solution is stored in the dark in an ice bath. After 30 minutes it is ready for use. The solution is standardized by using *m*-toluylene diamine (resublimed) or recrystallized 1-phenyl-3-methyl-pyrazolone-5. The solution of diazonium salt should not be used after 5 hours because of its instability.

0.1 *N* *m*-nitrobenzene diazonium chloride standard solution. In a 500-ml volumetric flask cooled to 5°C, 200 ml of a 0.25 *N* *m*-nitraniline stock solution is placed: (34.5 g of *m*-nitraniline, 100 ml of concentrated hydrochloric acid, and 100 ml of water are heated to bring about solution and diluted to a liter with warm distilled water). Next, 50 ml of 1 *N* sodium nitrite which has been cooled to 5°C is added rapidly. The resultant solution is diluted to 500 ml with water which has been cooled to 5°C. It should give a positive test for nitrous acid when tested with starch-iodide paper and is ready for use after standing for one or two minutes. The solution should be stored in an ice bath in the dark. The solution should be practically colorless and not yellow and it must not be more than slightly turbid. The solution is standardized in the same way as the *p*-toluene-diazonium chloride standard solution and should not be used after standing more than 5 hours.

Procedure. Standardization of diazonium solutions. About 0.6 g of resublimed *m*-toluylenediamine or about 1 g of recrystallized 1-phenyl-3-methyl-pyrazolone-5 is weighed accurately into a 600-

ml beaker. To this are added 150 ml of water, 100 ml of 2 *N* sodium acetate, and 10 ml of 1 *N* acetic acid. The solution is cooled to about 10°C.

The solution is agitated with a mechanical stirrer, and the diazonium standard solution is added from a burette equipped with a water jacket through which water is circulating at about 10°C. The burette or the glass jacket should be of amber glass to minimize any decomposition of the diazonium salt by light.

The diazonium standard solution is added as rapidly as coupling will take place. No excess of diazonium salt should be allowed to accumulate since there is danger of decomposition, which leads to high results. To test for excess diazonium salt in the solution, a few drops of the solution are placed on a piece of filter paper. About a centimeter away from the edge of the liquid mark are placed a few drops of an indicator solution (1% resorcinol containing 0.5 g of sodium carbonate; or 0.1% H acid [1-amino-8-napthol-3-6-disulfonic acid] solution containing 5 ml of 2 *N* sodium carbonate can be used to indicate excess diazonium salt). Where the two liquid portions meet on the filter paper, a color will develop if excess diazonium salt is present; no color will be noted if the diazonium salt is absent.

To test for excess coupling agent (*m*-toluylenediamine or 1-phenyl-3-methyl-pyrazolone-5) in the reaction mixture tetrazo dianisidine solution is used. First, 50 ml of 0.04 *N* dianisidine hydrochloride is tetrazotized exactly with 0.1 *N* sodium nitrite (use starch-iodide paper to indicate excess nitrous acid). A few drops of this solution are put on a piece of filter paper about a centimeter away from the edge of a sample of reaction mixture, also placed on the filter paper. If a color is developed, it indicates that unreacted coupling agent is present in the reaction mixture.

The diazonium standard solution is added in portions, depending on the rate of coupling with the particular compound contained in the sample. The reaction mixture is tested for excess coupling agent and diazonium salt after each addition. The additions of diazonium solution should be in increments of 0.25 ml when near the endpoint. The endpoint is taken as the point at which a negative test for coupling agent and a negative test for diazonium salt are obtained. When this point is reached, the burette is read, a few drops more of diazonium solution are added, and the solution is tested for diazonium salt. If a positive test is obtained, the

previous reading is taken. If the dye is water soluble and the color interferes with the spot tests, sodium chloride is added to salt out the dye and show a clear endpoint.

The samples are analyzed in the same manner in which the diazonium solutions were standardized except that the sample is substituted for the *m*-toluylenediamine or the pyrazolone. The sample should contain 0.003 to 0.005 mole of coupling material. Also, if the acetate-acetic acid buffer is not sufficiently alkaline for a satisfactory coupling rate, 100 ml of 2 *N* sodium carbonate can be used. The diazonium salt solution should be standardized in the presence of the same base as is needed for the sample. Sodium hydroxide can be used for materials which couple very slow or for samples soluble only in sodium hydroxide, but larger errors are more likely to be encountered when using this base, since the diazonium salt is readily decomposed by it.

$$\frac{\text{Milliliters of diazonium} \times N \text{ diazonium} \times \text{mol. wt. coupler} \times 100}{\text{Grams sample} \times 1000} = \% \text{ coupler}$$

To determine diazonium salts, a 50-ml aliquot of 0.1 *N* solution of *m*-toluylenediamine or 1-phenyl-3-methyl-pyrazolone-5 is added together with 100 ml of 2 *N* sodium acetate and 10 ml of acetic acid. The pyrazolone is the better since it couples in only one position while *m*-toluylenediamine may couple in two. The sample should contain 0.003 to 0.004 mole of diazonium compound. The excess coupling agent is then titrated with standard diazonium solution as above. The pyrazolone solution should be approximately 0.1 *N*, prepared with a weighed amount of recrystallized 1-phenyl-3-methyl-pyrazolone-5.

Calculations.

Milliliters of diazonium solution used on 50-ml aliquot of pyrazolone solution − milliliters of diazonium solution used on sample = A

$$\frac{A \times N \text{ diazonium solution} \times \text{mol. wt. diazonium salt} \times 100}{\text{Grams sample} \times 1000} = \% \text{ diazonium salt}$$

The procedure was tested with *m*-phenylenediamine, 1-naphthol-4-sulfonic acid; 7-amino-1-naphthol-4-sulfonic acid; 1- and 2-

naphthol; phenol; resorcinol; 2-naphthol-3-6-disulfonic acid; phloroglucinol, acetoacetanilide; 2, 3-dihydroxynaphthalene; 2-3 dihydroxynaphthalene-6-sulfonic acid.

The main precautions to be observed in this procedure are to avoid adding the diazonium solution too fast, to keep the temperature of the solutions below 10°C, and to avoid excess alkalinity. All these factors lead to decomposition of diazonium salts and high results.

General Reference

Eduard Fierz-David and Louis Blangey, *Grundlegende Operationen der Farben Chemie*, p. 238, Springer, Verlag, Vienna, 1943 (lithoprinted by Edwards Brothers, Ann Arbor, Mich., 1944).

23 Quantitative Separation of Components in a Mixture

CHEMICAL METHODS

Some organic compounds can be precipitated from mixtures. For instance, some molecules containing sulfonic acids, carboxylic acids, or phenolic hydroxyl groups can be precipitated with barium or silver. Unfortunately, samples to which such simple methods of separation can be applied are rare. However, in many mixtures the components of the mixture vary quite markedly as to water solubility or solubility in organic solvents, so that one component is readily extracted from the mixture by a solvent in which the other components are insoluble; or one component can be easily converted to a compound whose solubility is more adaptable to an extraction. For instance, if the sample were a mixture of phenol and a highly alkylated phenol or an alkylated naphthol or a hydroxy anthracene, the sample could be dissolved in benzene or toluene and the unsubstituted phenol could be quantitatively removed from the mixture by repeated extraction with water. The unsubstituted phenol could then be determined without interference from the other phenols in the mixture. Another example would be a mixture of an alcohol of a low molecular weight, such as ethanol, and an alcohol of a very high molecular weight, such as octadecanol. Such a mixture could also be separated into components by dissolving in benzene and extracting with water the alcohol of low molecular weight.

As happens more often, the components of the mixture do not vary enough in solubility to permit separation as just described. However, very often one component of the mixture can be converted to a form which has the desired solubility characteristics. For instance, if the mixture consists of an amine and a phenol, chemical analysis of such a mixture is difficult since both brominate and acetylate. However, the sample could be made acid with hydrochloric acid, thus forming the amine hydrochloride, and, on extraction with benzene or ether, the phenol could be separated

since only it is soluble in these solvents. The following conversions can be used to obtain desirable solubility characteristics for achieving separation: carboxylic and sulfonic acids and phenols can be converted to their sodium salts, which are very soluble in water and very insoluble in benzene; amines can be converted to hydrochlorides, which are very soluble in water and insoluble in benzene; the same applies to aldehydes and ketones, which may be converted to the bisulfite addition products.

FRACTIONAL DISTILLATION

One of the simplest and most widely used methods of separating components in a mixture is to distil them apart fractionally. For quantitative analytical purposes, it is not necessary to separate the component to be determined from all the other components; it is necessary to separate only the component to be determined from the component which interferes in the analysis.

This method of separation, however, is not foolproof. If the desired component and the interfering component boil only a few degrees apart, separation by distillation is difficult. Also, if the desired component and the interfering component form constant boiling mixtures (azeotropes), separation is impossible by distillation.

The ease of separation by distillation is dependent on the efficiency of the fractionating column used. Columns of 20 to 30 theoretical plates can readily be obtained from many manufacturers of laboratory apparatus. Fractionating columns of 100 theoretical plates can be obtained for more difficult separations (Podbielniak distilling apparatus manufactured by Podbielniak Inc., Chicago, Illinois).

Fractional distillation equipment capable of distilling samples as small as one milliliter can be purchased from Todd Scientific Company, Springfield, Pennsylvania. The efficiency of such equipment ranges up to about 50 theoretical plates.

DIALYSIS

Organic compounds can be separated by dialysis, a suitable membrane being used. A discussion of membranes is presented below in the section on electrodialysis, p. 123. If the difference in the molecular sizes of the organic materials in a mixture is great

enough, dialysis can be employed to achieve a separation of the components. This procedure is very slow but it is applicable in some cases. To speed the procedure, the water around the dialysis cell should be changed often. If the organic molecules are ionizable (sulfonates, carboxylates, etc.), electrodialysis should be the procedure used, since it is much faster.

The details for the dialysis procedure are identical with those for electrodialysis except that no electric current is used.

ELECTRODIALYSIS

This method can be applied to separate electrically charged organic molecules from neutral organic molecules. It can also be utilized for determining inorganic salts in organic materials.

INORGANIC SALTS CONTAINED IN MIXTURES WITH ORGANIC COMPOUNDS

Determination of inorganic salts in the presence of an organic compound is often very simply accomplished by ordinary inorganic analytical methods. However, occasionally the organic material will interfere with such determinations. For instance, sodium chloride or sulfate cannot often be determined in the presence of sulfonic acid groups by the usual inorganic methods, since both barium and silver frequently precipitate the sulfonic acid as well as the sulfate or chloride mixed with the organic compound.

This situation arises often where an organic chemical is salted out of solution with an inorganic salt. We also find this situation where commercial materials are diluted with inorganic salts and the finished product is a mixture of organic compound and inorganic salt. In some chemical reactions an inorganic salt is formed together with the organic compound and consequently the inorganic salt is found in the final product.

In a sample containing an inorganic salt mixed with organic compounds in which the organic compound contains no metallic atom but still interferes in the analysis for the inorganic material, the separation is rather simple. The sample is ignited and the organic matter is burned off, leaving the inorganic material untouched, or in some form which can be determined by inorganic analytical methods.

The samples requiring special attention are those in which the

organic material contains a metallic atom, in which case ignition is of no value whatever. In these cases electrodialysis methods are employed to separate the inorganic material from the organic matter. Membranes of different permeabilities are used, depending on the relative size of the inorganic ion to be determined and the size of the organic molecule or ion which is also present. The methods described here are general techniques. No specific procedure can be described, since each sample is different and has to be treated in accordance with its properties. For instance, in determining sodium sulfate in the presence of an organic compound whose molecules are very large a membrane with a rather great porosity can be used, since the organic molecule is large enough not to pass through the membrane. In the case of determining sodium sulfate in the presence of an organic compound whose molecules are rather small, a membrane of very fine porosity has to be used. When a coarse membrane is used, the electrodialysis goes to completion in a relatively short time; when the membrane is finer, the time for complete dialysis increases. When very fine membranes have to be used, a higher voltage must be applied in order to complete the process in a reasonable length of time.

Two organic molecules can be separated by electrodialysis, but there are restrictions. They are discussed at the end of this section on electrodialysis.

Apparatus (Fig. 19).

A and *B* are platinum electrodes.

C is the dialysis cell which consists of a piece of glass tubing, the diameter of which is chosen according to the amount of solution it will contain. Sealed to the base of *C* is the membrane *D*. The seal should be free of leaks. Collodion, sealing wax, and rubber cement are suitable to seal the membrane onto the glass tube; collodion is the most generally applicable. For a source of d-c current, a storage battery, small d-c generator, or a rectifier on an a-c line can be used.

Membranes. There are four materials which make good membranes: cellophane, collodion, rubber, and parchment. Cellophane itself is a membrane of very fine porosity. It can be used for separating inorganic ions from organic compounds with small molecules, but it is a very "slow" membrane. Cellophane can be made more porous by swelling it with calcium thiocyanate. The increase in porosity can be controlled by varying the concentration of the calcium thiocyanate and the time of immersion. The

thiocyanate is removed from the membrane by repeated soaking in water.

Collodion makes a good membrane for general use. The thickness of the membrane can be controlled by varying the concentration of the collodion solution used in the preparation of the membrane. The thinner the membrane, the "faster" it is. The porosity of the membrane can be varied by varying the composition of the solvent mixture and rate of drying and also by incorporating a little glycerol in the solution. The presence of glycerol causes the formation of membranes of higher porosities.

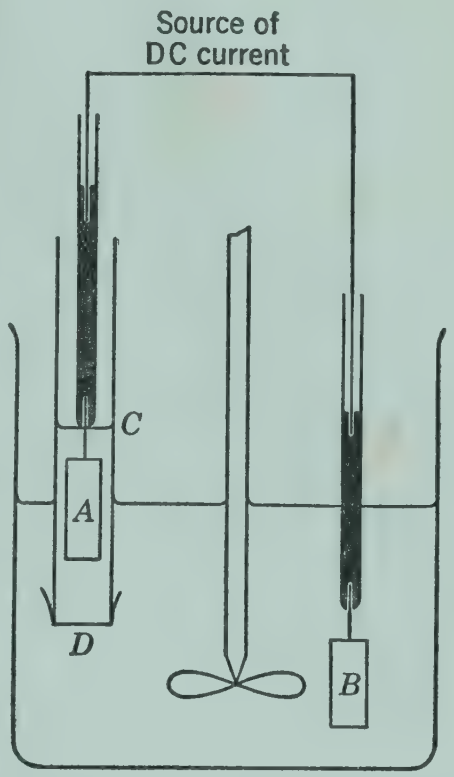


FIGURE 19. Electrodialysis apparatus.

To prepare a collodion membrane of the desired porosity it is necessary first to prepare a collodion solution of the proper concentration. The proper concentration is found by trial and error but, once it is established, membranes having the same porosity are readily prepared. A piece of Kleenex or lens paper is sealed over one end of the piece of glass tubing which is to act as the dialysis cell. The tissue can be sealed to the glass tubing with ordinary collodion, sealing wax, or a rubber cement and as shown in Fig. 20.

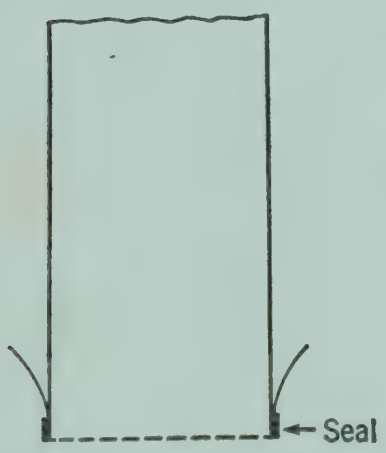


FIGURE 20. Dialysis cell.

The tissue-paper covered end of the glass tubing is then immersed in the collodion solution; the tissue paper should be immersed over the seal so that all exposed parts of the paper are covered. The tissue paper is not allowed to stand in the collodion; it is just dipped and removed. The excess collodion solution which adheres to the tissue when it is removed from the solution is removed by tilting the dialysis cell to an angle of about 45 degrees and touching the edge of the membrane to the surface of the solution. The membrane is then allowed to dry at the desired rate.

Rubber membranes can be made from most types of very thin rubber sheeting. Very fine membranes can be made from rubber finger cots (used to protect wounds on fingers). Untreated finger cots are practically impermeable to inorganic ions; however, by swelling in methanol-benzene solutions the porosity can be increased. The increase in porosity depends on the time of immersion in the swelling solution and the concentration of benzene in the solution. The finger cot can be used as the dialysis cell without cementing it to a glass tube. Other types of rubber sheeting, such as rubber pants for babies and surgeon's rubber gloves, can also be used for membranes.

Parchment membranes, which can be purchased in varying porosities, work very well as dialysis membranes. The porosity of parchment, like the porosity of cellophane, can be increased with calcium thiocyanate.

To test membranes for their permeability toward the inorganic ion and their impermeability toward the organic molecule, a mixture is made of an organic dye or colored material whose structure most closely resembles the structure of the organic molecule contained in the sample. To this colored organic material is added a small amount of an inorganic material with the ion to be determined. This mixture is put in a dialysis cell with the membrane to be tested, and the voltage is applied as described in the procedure below. If no color comes through the membrane, it signifies that the membrane can hold back the organic molecule. The liquid outside the dialysis cell is tested semi-quantitatively from time to time for the inorganic ion to show how rapidly the inorganic ion premeates the membrane. If none of the inorganic ion is detected outside the membrane after 2 hours, the membrane is either impermeable or is too "slow" to be practical, and a more porous membrane is prepared. When a membrane is found which holds back the colored organic material but allows the inorganic ion to pass through at a reasonable rate, a synthetic sample is made up using the organic compound and the inorganic salt contained in the samples to be analyzed. These synthetic samples are dialyzed for various lengths of time, and the efficiency of the membrane is thus tested under the exact conditions encountered in the actual analysis. Any modification of the membrane can then be applied more exactly before the actual samples are run.

Procedure. The sample is placed in the dialysis cell in water solution. The cell is immersed in about 100 ml of water contained in a 250-ml beaker. If the difference in particle size between the organic molecule and the inorganic ion is large, the dialysis cell is immersed in the water so that the level of the sample solution in the cell is higher than the level of the water in the beaker. This hydrostatic pressure in the dialysis cell causes some ultra-filtration of the sample through the membrane, which augments the effect of the electric voltage in bringing about the separation of the inorganic ion from the organic molecule. If, however, the size of the organic molecule is close to the size of the inorganic ion, it is best to have the level of the sample in the dialysis cell below the level of the water in the beaker. In this way the back pressure helps to prevent any migration of the organic molecules through the membrane, but the electric current acting on the inorganic ions can pull these ions through the membrane in spite of the back pressure.

When the dialysis cell containing the sample is in the water, a platinum electrode is inserted in the sample solution in the cell and another electrode is inserted in the water. The water should contain an electrolyte which will not interfere in the final analysis. The polarity of the electrodes should be such that if the negatively charged ion of the inorganic salt is to be determined the anode is in the water layer in the beaker; if the positively charged ion of the inorganic salt is to be determined, the cathode should be in this water layer. The voltage is applied for a predetermined length of time to give complete separation for that particular sample and membrane. The water in the beaker is then analyzed for the ion in question.

When the ion to be determined can plate out as a gas on the electrode in the water layer (such as the halide ions), a special electrode has to be used (see Fig. 21). In this case any gas plating out on the electrode passes through *E* and bubbles through beaker *F*, which contains a material to absorb or react with the particular material being determined. When halides are determined, a small beaker of 50% potassium hydroxide is used as a trap, and any halogen liberated at the electrode is absorbed chemically by the alkali. For the final determination of halide, the contents of the trap are combined with the water in the beaker, acidified, and analyzed by a Volhard titration.

If the dialysis requires a long time, it is advisable to use a smaller amount of water in the beaker and to change the water from time to time. In this way the concentration of ions in the beaker is always much less than the concentration of ions in the dialysis cell, and the diffusion of ions out of the dialysis cell is greater.

The above procedure was used in determining sodium sulfate and chloride in detergent and dye samples and was found to work

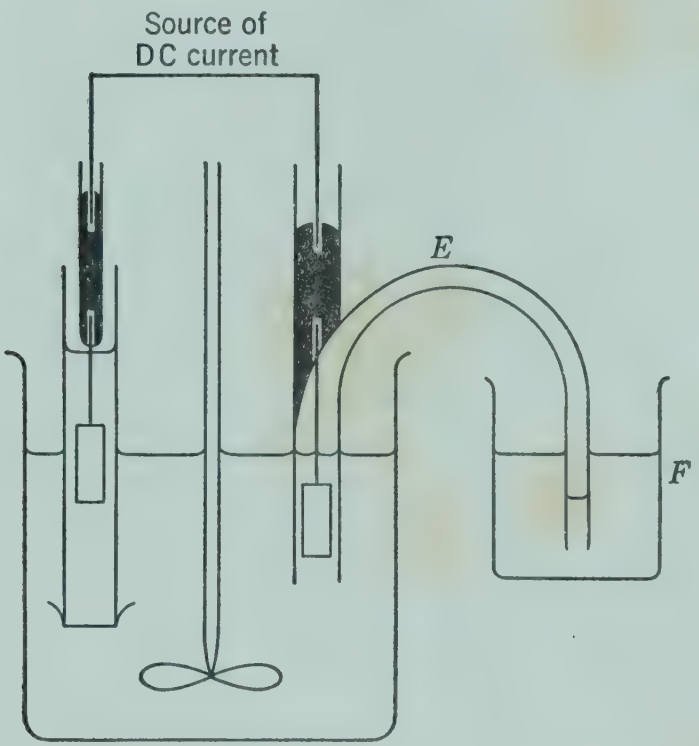


FIGURE 21. Electrodialysis apparatus.

very well. The time for the electrodialysis varied with the magnitude of the applied voltage, the porosity of the membrane, and the amount of inorganic material in the sample. The sample requiring the longest time completely to separate the inorganic salt from an organic compound was a sample containing 10% sodium sulfate in malachite green. This sample required 48 hours. A collodion membrane was used, and the source of d-c current was a 6-volt storage battery.

Results obtained, by determining standard mixtures of inorganic salts in dyes and detergents, were accurate to $\pm 1\%$ of the theoretical amount of inorganic salt contained in the sample. The precision of the determination was of the same magnitude.

SEPARATION OF ORGANIC MOLECULES BY ELECTRODIALYSIS

Two organic molecules can be separated by this method provided that there is sufficient difference between the molecular sizes and that one of the organic materials contains a functional group of sufficient polarity to be drawn to the electrode of opposite polarity. Sulfonic and carboxylic acids and phenols can be drawn through a membrane with the aid of an electrode. If the difference between the molecular sizes of the organic compounds is small, electrodialysis will not achieve a clean separation. Also, if both organic molecules are charged alike, separation is also difficult.

It must be remembered that hydrogen is liberated at the cathode and oxygen at the anode; therefore, reduction can take place at the cathode and oxidation at the anode. If the substance to be determined is readily reduced and is to be collected at the cathode, it may be reduced by the hydrogen liberated at this pole. Dyes, for instance, will be seen to fade rapidly at the cathode because of their reduction.

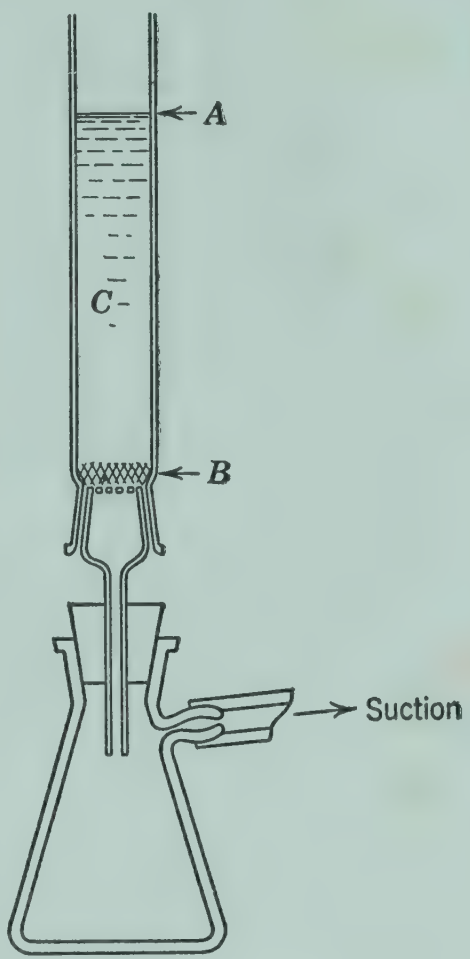


FIGURE 22. Chromatographic adsorption apparatus.

CHROMATOGRAPHY

Mixtures of some organic compounds, especially if they are colored, can be resolved by chromatographic adsorption methods. The separation in this case depends on the difference in the adsorption characteristics of the components of the mixture toward a given adsorbent. Mixtures of colorless materials can also be resolved, but they are more difficult, since the adsorption bands are invisible.

The procedure consists first, of packing a glass column with an adsorbent (see Fig. 22). Alumina, silica, silicic acid, and talc are the most commonly used adsorbents. In Fig. 22, *C* represents the adsorbent; *B* is a glass wool plug to prevent loss of adsorbent from the column; *A* is a disk of filter paper placed on top of the

adsorbent to prevent the surface of the adsorbent from being disturbed when the solution is poured into the column. Next, solution of the sample is poured through the column, and the components are resolved by elution with various solvent mixtures.

A typical chromatographic adsorption can be described as follows. The column is packed as in Fig. 22 with a suitable adsorbent for the particular organic compounds being used. After the adsorbent is added to the chromatographic tube, the tube should be tapped on the table top to assure homogeneity of the adsorbent throughout the column. If the adsorbent is not packed homogeneously in the column, the rate of flow of the solution will be irregular in different sections of the column, and irregular bands

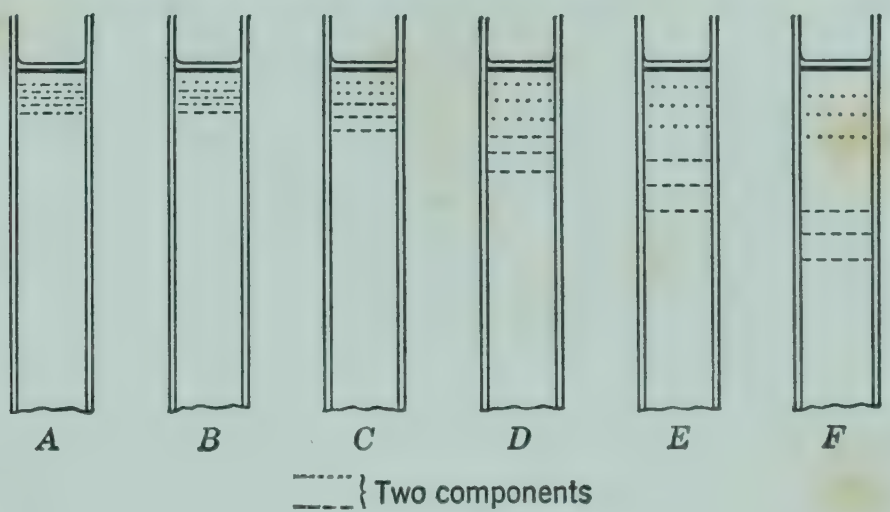


FIGURE 23.

will be obtained, both of which are undesirable for quantitative separations. The adsorbent should be such that it adsorbs one component more strongly than the other from a certain solvent or solvent mixture. The sample should be dissolved in a solvent from which the organic compounds are very easily adsorbed. The solution is then poured onto the adsorbent. If the adsorbent is coarse, gravity provides a fast enough flow; if the adsorbent is finely divided, slight suction can be applied on the flask to obtain a suitable rate of flow. If the adsorbent very readily adsorbs components of the particular system being studied, a coarse-grained adsorbent is suitable. A fine-grained adsorbent is used when a higher adsorptive strength is desired.

As the sample solution passes through the column, the components of the sample should be adsorbed by the adsorbent at the top of

the column (see Fig. 23). This is indicated by the coloring of the adsorbent at the top of the column. Then various selected solvents or solvent mixtures are run through the column in order to desorb one of the components from the adsorbent. This is accomplished by the difference in the adsorption tendencies of the adsorbent toward the various components. The component desorbed more rapidly is concentrated in the solvent and is readsorbed further down the column (see *B–D* Fig. 23). For a quantitative separation the bands indicating the different components should be completely separated (see *F* Fig. 23). The column should not be allowed to "run dry." This means that the liquid surface above the adsorbent should not be allowed to disappear before the next addition of solvent or solutions. As the surface of the solution approaches the surface of the adsorbent, fresh solvent or solution is added until the chromatogram is complete.

After the chromatogram is complete, the adsorbent plug is pushed out of the tube by means of a convenient rod and the section of the adsorbent containing the desired component cut off and transferred, as quantitatively as possible, to a flask. The component is then removed from the adsorbent by successive extractions with a convenient solvent, and the resulting solution is analyzed for the component. Chromatographic methods were used to separate various dye mixtures, and separations better than $\pm 5\%$ of the original composition of the mixture were often obtained.

If there are colorless components in the mixture, the procedure is more difficult since the bands are invisible. Then the plug of adsorbent is pushed out of the chromatographic tube, and a reagent which forms a color with the component is painted on the adsorbent plug. In this way, the time required for complete separations, for that particular system, is determined. Then the sample is run for this predetermined length of time. The disadvantages of chromatography for quantitative separations when the components of a mixture are colorless are obvious. Trial and error have to be used to determine the time for complete reaction, and then we are never absolutely sure that the separation was complete, with no loss of the desired component. Optical methods (refractive index) are now available to indicate the separation of the various components by means of chromatographic adsorption. Such

methods are described in detail in a booklet by Stig Claesson, *Studies on Adsorption and Adsorption Analysis with Special Reference to Homologous Series.* This booklet is published in London by H. K. Lewis and Company, Ltd., 136 Gower Street, and in Paris by the Librairie C. Klinchsieck, 11 Rue de Lille.

The above is just a skeleton procedure. Nothing has been said, for instance, about choice of adsorbent or choice of solvents. It is very difficult to go into too much detail in a book of this sort since the treatment of each different sample requires a different approach. For persons interested in chromatographic methods of separation, the textbook *Principles and Practice of Chromatography,* by L. Zechmeister and L. Cholnoky (translated by A. L. Bacharach and F. A. Robinson), John Wiley and Sons, 1941, is recommended.

ELECTROPHORESIS

Electrophoresis is defined as the migration of charged particles under the influence of an electric field. Since polar organic molecules seldom have electrical charges of the same sign and same magnitude, electrophoresis provides a means of separating such organic compounds in a mixture.

In electrophoresis, the speed of the separation depends on the magnitude of the applied voltage, the concentrations of the components, the charges on components, and the medium used for the separation. Electrophoresis is more advantageous than electrodialysis or dialysis for separating organic molecules since the preparation of a suitable membrane for dialysis is very time consuming. Also, electrophoresis can be used to separate mixtures when the molecular sizes of the components are not different enough for dialysis or electrodialysis, and, in addition, electrophoresis is faster and yields a cleaner separation than dialysis or electrodialysis.

Strongly charged organic molecules such as carboxylic and sulfonic acids and their salts, as well as amines and phenols, are very easily separated from other organic compounds, since most other organic compounds are very weakly charged.

Because electrophoresis procedure described below is just a skeleton procedure, some modifications to handle particular samples may have to be made to make the procedure applicable.

Apparatus. (Fig. 24.)

A = platinum electrodes in electrolyte solution.
B = medium used.
C = filter paper disks.
D = sample.
E = sintered glass disks.

Procedure. There are various satisfactory media for electrophoretic separations; among them are gelatin, silica gel, alumina, talc, silicic acid, and calcium carbonate. Gelatin is a very slow

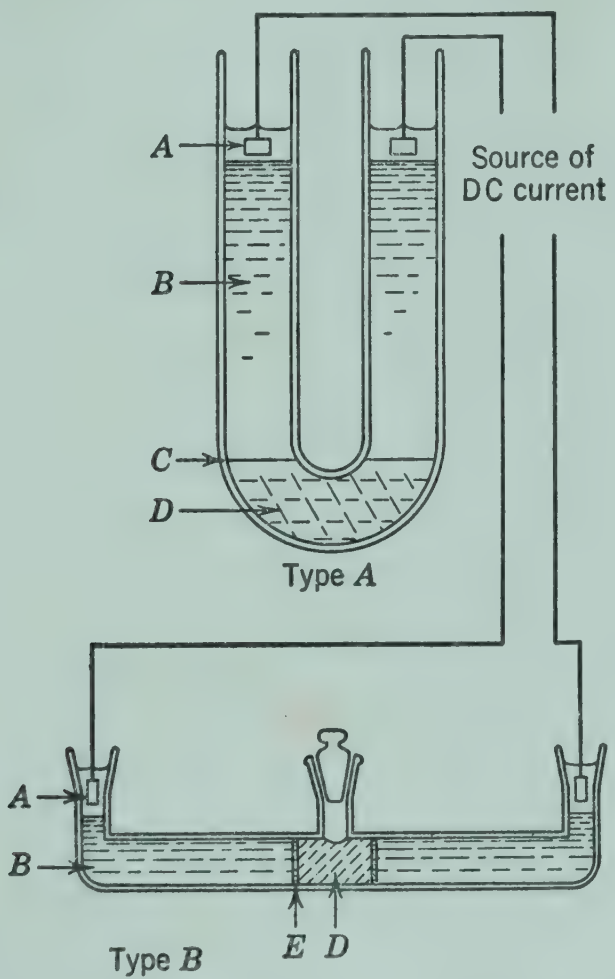


FIGURE 24. Electrophoresis cells.

medium; the speed of particle migration in the solid media depends on the grain size of these media. The advantage of the solid electrophoresis media is that non-aqueous as well as aqueous solutions can be used, whereas gelatin can be used only for aqueous solutions. Also, a solid medium may be chosen whose adsorption effects on the components of the sample may augment the action of the electric current in achieving faster and cleaner separations.

The sample is first dissolved in a convenient solvent. Most

solvents are satisfactory except very non-polar solvents, such as petroleum ethers, ethers, and benzenes. A suitable electrolyte is also dissolved in the solution; it can be any acid, base, or salt which will dissolve in the solvent and not react with the sample or interfere with the final analysis. The sample solution is then put in the electrophoresis cell.

Type A—Electrophoresis Cell (*see Fig 24*). The sample is introduced into the bottom of the cell by means of a long-stemmed funnel. Care should be taken not to get any of the sample on any other section of the cell, or else the final desired fraction may be contaminated by this material. When a gelatin medium is used, the sample solution contains both gelatin and the electrolyte. The solution is introduced into the cell as described above, and the gelatin is allowed to cool and set. Then the gelatin solution, which is to act as the electrophoresis medium (indicated by *B* in Fig. 24), is poured into the side arms of the electrophoresis cell. The gelatin solution should be cool when added and should be poured gently so as not to upset the solid gelatin plug containing the sample. An electrolyte should be dissolved in the gelatin to conduct current. It is best to pour a little gelatin at a time into each arm of the cell, since if one arm of the cell is filled with solution before the other, the hydrostatic pressure may be great enough to push out the plug of sample.

In using solid electrophoresis media essentially the same procedure is followed. A slurry is made of the medium and the solution of the sample and electrolyte. The consistency of the slurry should be about that of a rather thin whipped cream. This slurry is introduced into the bottom of the cell, by means of a funnel with a long, rather wide stem to avoid getting any sample on the side arms of the cell. A disk of filter paper (*C*) is then put on each surface of the slurry containing the sample, and a slurry containing only the solid medium and the electrolyte is then added gently. The filter paper disks aid in preventing any disturbance of the sample plug. Again, in using the solid media, a little slurry at a time is added to each arm to prevent the sample containing slurry from being displaced by hydrostatic pressure.

With the cell filled, a few milliliters of electrolyte solution are then added to the surface of the material in each arm. If the platinum electrodes are immersed in the slurry itself, the gases released at the electrodes cause gas pockets in the slurry around

the electrodes and the efficiency of the contact is decreased. The electrodes should be placed in the electrolyte solution above the slurry.

After the electrolytic separation is complete, the plug of electrophoresis medium is pushed out and the bands are separated. The desired band can be removed with a long thin spatula. The material is dissolved away from the medium, and it is determined by using the methods described in this book.

Type B Cell (see Fig. 24). Of the two types, this cell is the more convenient to use. Since it is in a separate cell, the sample can be easily introduced, and it will not be disturbed when the arms of the cell are filled with the electrophoresis medium. Also, when two materials of opposite charges are being separated, each component will go into a different arm and the separation is simple. When the two components are charged alike, the two bands will appear in the same arm, one traveling faster than the other. As soon as clear medium is noted between the bands, the bands can be separated as discussed for the type *A* cell.

Because no membranes are needed and because the time required for complete separation of components in a sample is about half, electrophoresis is better than electrodialysis. Also, materials of the same molecular size can be separated, provided that the charges on the molecules are of different sign or magnitude. Colorless mixtures cause difficulty, as in chromatography, since the bands cannot be viewed. However, the same technique can be used as in chromatography (see pp. 131–132); also cells exist which are equipped to handle colorless samples. A complete discussion of various electrophoresis cells used to separate proteins which can be applied to other organic compounds is given by D. A. MacInnes and L. G. Longsworth in Jerome Alexander's *Colloid Chemistry*, Vol. V, pp. 387–411, Reinhold Publishing Corporation, 1944. The same optical methods mentioned on pp. 131–132 for chromatography can be used for electrophoresis.

The Klett Manufacturing Company, 179 East 87 Street, New York City, the American Instrument Company, Silver Springs, Maryland, and the Perkin-Elmer Corporation, Glenbrook, Connecticut, manufacture electrophoresis apparatus. Literature on the various types available can be obtained from them.

24 Some Instrumental Methods Applicable to Quantitative Analysis

REFRACTIVE INDEX AND DENSITY

These physical measurements provide a rapid and precise way to determine the amount of materials present in a two-component system. Samples containing more than two components cannot

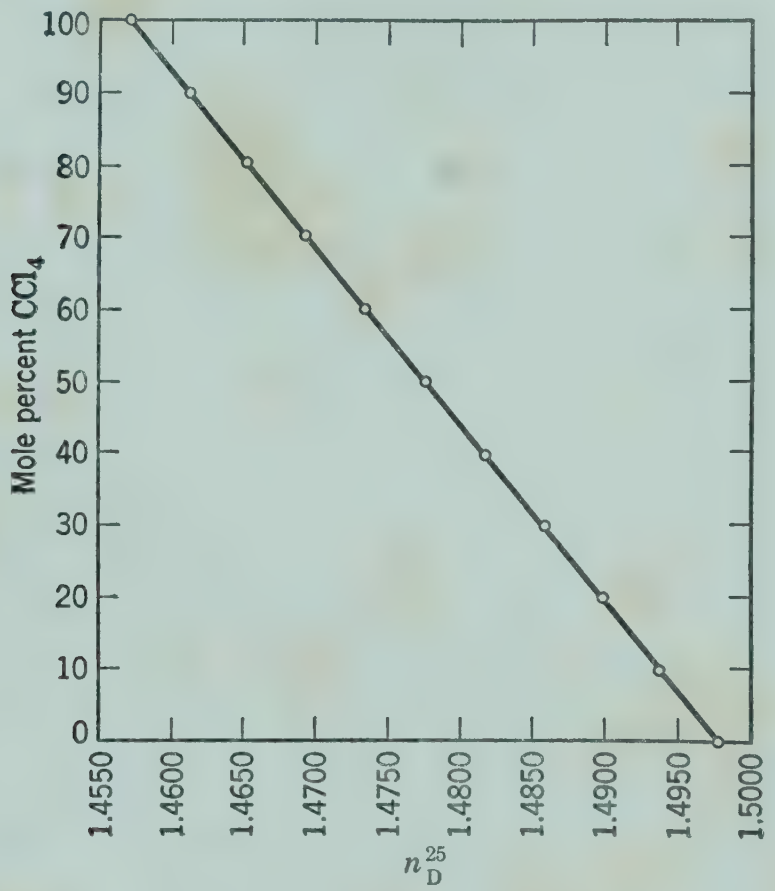


FIGURE 25.

be determined by these methods. Refractive index and density can be applied when these physical constants for the pure components of the mixture are sufficiently different. For instance, a system of *n*-butyl alcohol and *n*-butyl vinyl ether could not be analyzed by refractive index measurement since the refractive index of *n*-butanol (n_D^{20}) is 1.3993 and the refractive index of the ether is 1.4022 (n_D^{20}). The difference between the two indices is

0.0029, and since a refractive index measurement is usually only precise and accurate to ±0.0002, an error of 10% can be made just in reading the instrument. However, density measurements could be used in this case since the density of *n*-butanol is 0.8062 g/ml (25°/4°C) and the density of *n*-butyl vinyl ether is 0.7733 g/ml (25°/4°C). The difference in densities is 0.0329 g/ml, and, since density measurements can be obtained to at least ±0.0002,

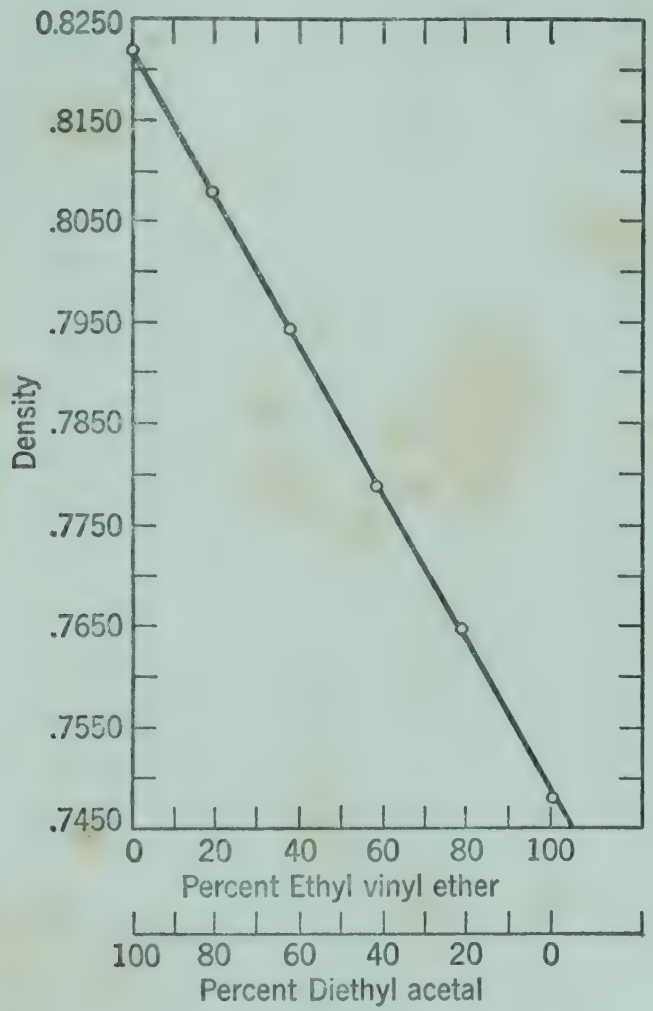


FIGURE 26.

results can be obtained with an accuracy and precision of about ±1%. When the difference between the physical constants of the two components is greater, the results are more accurate. For instance, in a system of propargyl alcohol ($n_D^{20} = 1.4306$) and water (n_D^{20} 1.333). The difference between the refractive indices of the components in this system is 0.0976, and, since refractive index measurements are good to ±0.0002, results can be obtained to ±0.3%.

For carrying out actual determinations, a calibration curve of

refractive index or density versus percentage composition is necessary. The calibration is done by making up known mixtures of the two components and measuring the refractive index or density and plotting the calibration curve. Figure 25 shows a typical calibration curve for refractive index versus composition in a system of carbon tetrachloride-benzene. Figure 26 shows a typical curve for density versus composition for the system of ethyl vinyl ether-diethyl acetal. The refractive index or density of the unknown sample is then taken, and the percentage composition is read off the calibration curve.

POLARIMETRY

Some compounds have the property of rotating the plane of polarized light passing through them. The degree of rotation is a function of concentration as expressed by the equation

$$(\alpha)_t^\lambda = \frac{\alpha}{l(g/V)}$$

where $(\alpha)_t^\lambda$ is the specific rotation at temperature t and wavelength λ; α is the observed angle of rotation; l is the length solution traversed by the light; and g/V is the concentration of the optically active component.

Compounds which contain an asymmetric carbon (or other atoms) and which therefore rotate the plane of polarized light can be determined in a mixture in which the other components do not affect the plane of polarized light.

The procedure consists of preparing a solution of the sample in an optically inactive solvent and measuring the degree of rotation in a polarimeter. The percentage of the optically active component can be calculated from the above equation, the specific rotation having been predetermined by known mixtures of the optically active component. A calibration curve prepared in the same fashion could be used.

Although the above method is the standard one for determining sucrose in water solutions, it can very readily be applied to determining any optically active compound present in a mixture in which the other components are optically inactive.

Many compounds which are normally optically inactive can be

made optically active by placing them in a magnetic field. Complex mixtures cannot be analyzed by this method since practically all the components will become optically active in the magnetic field and will contribute to the total rotation. This method is limited to two component systems in which the difference in induced optical activity of the two components is sufficiently large to provide accurate analyses.

This induced optical activity is known as the Faraday effect. Induced optical activity differs from ordinary optical activity in that in the latter, if the light is reflected back along its course, after having passed through the sample, the resultant rotation will be zero. In magnetically induced rotation, the direction of rotation depends upon the direction of the magnetic field; since this is the same for the light passing in both directions, reflecting the light back through the sample will result in double the original effect.

The angle of induced rotation (α) is proportional to the length of sample traversed by the light (L), and to the strength of the magnetic field (H):

$$\alpha = WLH$$

where W is known as the Verdet constant, which is characteristic of the material being studied.

In using this physical property as a quantitative analytical tool a calibration curve has to be established. Known mixtures of the two components are prepared and the rotation induced by the magnetic field is measured. Rotation versus concentration is then plotted.

As in all the previously mentioned physical measurements, the accuracy and precision of this method depend upon the magnitude of the difference between the Verdet constants for the two components. If the constants for the two components are too similar, this method of analysis cannot be applied. The greater the difference between the Verdet constants for the two components in the sample, the greater will be the accuracy and precision of the analyses. The accuracy and precision also depend on the sensitivity of the polarimeter being used. Temperature affects the induced rotation and the effect of temperature on the system should be determined. If the effect is significant, appropriate temperature control should be applied.

DIELECTRIC CONSTANT

This physical constant can be used to determine the constituents in two component systems. The determination of dielectric constant consists of the measurements of the electrical capacity of a condenser filled with the sample. This electrical capacity is compared to the capacity of the condenser when there is a vacuum between the plates.

Here, a calibration curve prepared by using known mixtures of the components occurring in the mixtures is necessary for quantitative analytical work.

LIGHT ABSORPTION

The wavelengths of light absorbed by a sample are characteristic of the compounds contained in the sample. The degree of absorption is dependent on the concentration of the material doing the absorbing. This makes possible the use of light-absorption measurements as quantitative measures of concentration. The mathematical relationship between light absorption and concentration is known as the Beer-Lambert law:

$$I = I_0 e^{-KlC} \tag{1}$$

where I is the intensity of the light transmitted through the sample, I_0 is the initial intensity of the light, K is the absorption coefficient of the material being studied (this constant is characteristic of the material), l is the length of sample through which the light is passed, and C is the concentration of the absorbing component. The equation can be modified to a straight-line relationship as follows:

$$\frac{I}{I_0} = e^{-KlC} \tag{2}$$

where I/I_0 = fraction of light transmitted. If the logarithm (base 10) of both sides is taken,

$$\log \frac{I}{I_0} = -ElC \tag{3}$$

where E is a new constant known as the extinction coefficient ($E = 2.3K$).

$$-\log \frac{I}{I_0} = ElC \tag{4}$$

$-\log I/I_0$ is designated as the optical density of the solution (D).

$$D = ElC \tag{5}$$

When a certain compound in a mixture is to be determined, curves of density versus wavelength are obtained for pure samples of all the components of the mixture. A specific wavelength is then chosen for use in the quantitative measurements. This wavelength of light should be absorbed only by the compound to be determined. If another component of the mixture absorbs light of the same wavelength, a more complicated calculation is necessary, a process described later. Once a wavelength is found that is characteristic of only the compound to be determined, the extinction coefficient for that compound at that particular wavelength is determined. This is done by measuring the density of known solutions of the compound. The densities are plotted against the corresponding concentrations and, if the resultant line is straight, the compound obeys the Beer-Lambert law at that wavelength, and the slope of the line will yield the extinction coefficient. In analyzing unknown mixtures, the density of the sample is measured; and by substituting the predetermined extinction coefficient and the length of sample traversed by the light in equation 5, the concentration of the material of interest can be computed.

The instrument used to measure the intensity of the transmitted light is a spectrophotometer. A spectrophotometer consists of a polychromatic source of light which is broken up into its component wavelengths by means of a prism (diffraction gratings are also used). By the use of a slit, light consisting of a very small range of wavelengths is passed through the sample at one time. The wavelength of light passing through the sample is varied by rotating the prism. The intensity of the transmitted light is measured with a photoelectric cell. This intensity is compared with the intensity of the light passing through a blank solution, the difference between the two intensities yielding the absorption by the sample. Most commercially available spectrophotometers are calibrated to read both per cent transmission and density directly in order to facilitate quantitative work. Among the most popular spectrophotometers are the Beckman Quartz spectrophotometer, which covers a portion of the ultraviolet and infrared ranges and the visible range (about 220 to 1100 mμ); the Hardy Type General Electric spectrophotometer, which covers the visible

range of 400 to 700 mμ; and the Perkin-Elmer infrared spectrophotometer, whose range varies with the material used for the prism. The following table shows the range for each material.

Prism Material	*Range, microns*
LiF	2.5 to 5.5
NaCl	2.5 to 15.0
KBr	10 to 25

With each sample a blank must be run consisting of everything contained in the sample except the varying components.

When there are two or more components in the mixture whose concentrations vary, and both components absorb in the same wavelength range, the problem is somewhat more complex. If the concentration of one of the compounds is known, the blank can be made up containing that amount, and the difference in absorption between sample and blank will be due solely to the other component. If one component has an absorption which is characteristic of itself alone, this wavelength can be used for the quantitative determination, since the other component will not affect the readings regardless of its concentration in the mixture. When the concentrations of both components are unknown, and both absorb in the same range, special calculations have to be applied.

In a two-component system, the density measured at a certain wavelength is equal to the sum of the densities due to each component. For a certain wavelength this can be expressed.

$$D_{T_1} = D_{A_1} + D_{B_1}$$

where D_{T_1} is the density of the total sample at wavelength 1; D_{A_1} is the density at wavelength 1 if component A were present alone, and D_{B_1} is the density if component B were present alone. From the Beer-Lambert law we have seen that for single-component systems

$$D = ElC$$

If the same size cell is used for each determination, then the length term can be included in the constant so that the equation reads

$$D = eC$$

By substitution

$$D_{T_1} = e_{A_1}C_A + e_{B_1}C_B \qquad (6)$$

At a second wavelength the extinction coefficients for the two components change, and the expression reads

$$D_{T_2} = e_{A_2}C_A + e_{B_2}C_B \tag{7}$$

There are now two equations containing the concentration terms for components A and B. The densities at the two wavelengths on the total sample are the values read on the spectrophotometer scale. The extinction coefficient value (with the length term included in it) for each component at each wavelength has to be predetermined. The extinction coefficient values are measured by making up known solutions of each component. The densities of the solutions of each component are measured at the two wavelengths being used in the determination (the wavelengths chosen for the analysis are usually the wavelengths yielding maximum absorption by the sample). A curve of density versus concentration is then plotted for each component at each wavelength. The line should be a straight line if the material obeys the Beer-Lambert law, the slope of the line yielding the extinction coefficient value for the particular component at the particular wavelength. For the analysis of a two-component system, four extinction coefficient values are needed, one for each component at each of two wavelengths.

The extinction coefficient values are then substituted in equations 6 and 7 and, the densities of the sample at the two wavelengths having been measured, the concentrations of the components A and B can be calculated by solving the equations simultaneously.

A three-component system can also be analyzed by this method. In this case there are three simultaneous equations to be solved. Density measurements have to be made on the sample at three wavelengths, and nine extinction coefficient values have to be determined (one for each component at the three different wavelengths being used).

Systems involving more than three components can be analyzed, but the calculations become quite involved. Also, the accuracy of the method for more than two components drops off sharply because of accumulation of slight deviations from the Beer-Lambert law; also, the presence of any impurity not known to be in the mixture affects the more complicated system to a greater extent.

Quantitative spectrophotometric analysis is very widely used.

The method can be applied only when all the constituents in the mixture are known, since any material whose presence is unsuspected can absorb in the same wavelength range in which the analyses are being run and can therefore cause erroneous results.

In general, the simpler the system the more accurate will be the spectrophotometric analysis.

Texts which cover this material in much more detail and give useful details as to quantitative methods are *Optical Methods of Chemical Analysis*, by Thomas R. P. Gibb, McGraw-Hill Book Company, New York, 1942, and *Chemical Spectroscopy*, 2nd Ed., by Wallace R. Brode, John Wiley and Sons, New York, 1943.

POLAROGRAPHY

Polarography is a widely used tool for quantitative analysis. The book *Polarography*, by I. M. Kolthoff and J. J. Lingane, Interscience Publishers, New York, 1941, describes the use of this tool very ably and very completely.

General Reference

SAMUEL GLASSTONE, *Textbook of Physical Chemistry*, pp. 597–599, D. Van Nostrand Co., New York, 1940.

25 Weighing of Volatile or Corrosive Liquids

Need often arises for the determination of liquid samples which are highly volatile. These liquids cannot be weighed even in glass-stoppered weighing bottles without serious losses. Some liquids give off vapors or gases of a corrosive nature so as to cause possible injury to the balance being used.

A technique has been devised whereby the sample is sealed in a glass ampoule so that there can be no loss of vapors. Samples boiling as low as 0°C can be successfully weighed by this technique.

The preparation of the ampoule is described in Fig. 27. The bulbs are blown as shown in steps 1 to 4. Then the arm of the bulb is bent, as seen in step 5; and the making of the constriction is seen in step 6.

The design of the bulb makes possible the removal of a sample from a bottle (step 7) without awkward tilting of the sample bottles. The constriction makes possible a very quick seal (step 8) without significant vaporization of sample. Vaporization of more volatile components of a mixture can result in an unrepresentative sample.

These are the steps in taking a sample. The bulb of the ampoule as seen in step 6 is warmed gently in a flame to drive out some of the air by expansion. The tip of the capillary is then quickly inserted below the surface of the sample. The bulb of the ampoule is immersed in a beaker containing a mixture of dry ice and methanol or acetone. The contraction of the air in the bulb will draw the sample into the bulb. As soon as the flow of sample into the bulb has diminished, but not yet ceased, the tip of the capillary is withdrawn from the sample bottle. The sample in the capillary side arm is allowed to be drawn past the constriction. The bulb of the ampoule is kept in the dry ice bath while the ampoule is sealed. The sealing is accomplished by placing the constriction in the arm of the ampoule in the flame of a micro burner or in the flame produced by a Bunsen burner with the chimney removed. The flame can be brought to the edge of the beaker of dry ice and solvent without the contents of the beaker catching fire. This is probably due to the extremely low temperature of

the solvent and the atmosphere of carbon dioxide over its surface. To be safe, the beaker is held with a pair of tongs. This procedure is more comfortable since the beaker is extremely cold. The liquid contained in the capillary side arm which now is separated from the bulb (step 9) is driven out by holding the capillary in a

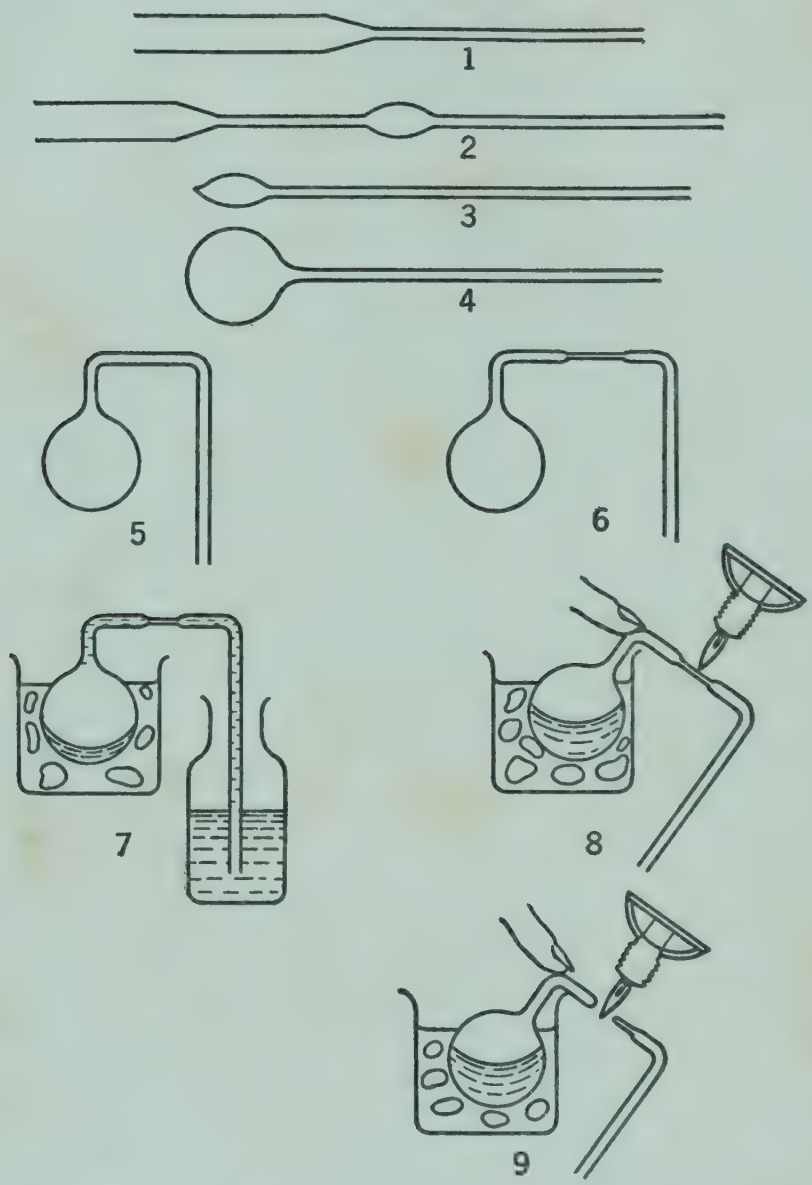


FIGURE 27. Ampoules for weighing volatile liquids.

flame with a pair of tweezers. This liquid should be entirely removed since the capillary side arm has to be weighed with the full ampoule because it was included in the weight of the empty ampoule.

A few precautions in sampling liquids are:

(1) No vapor should be lost from the bulb at any time. The sample should be drawn up in one operation. Do not try to draw up additional sample by warming the sample already in the bulb

and then recooling the bulb. This procedure would result in driving off the lower boiling portion of the sample and obtaining an unrepresentative sample.

In this same respect error in sampling can result if the bulb has been heated to too high a temperature for that particular sample. The liquid first striking the hot bulb can be partially vaporized off; this is indicated by bubbles escaping from the tip of the capillary as soon as the first liquid hits the bulb.

(2) Support the bulb with the fingers as the constriction is being sealed so that the bulb will not fall over into the cold alcohol or acetone bath. If the hot tip strikes the cold bath, cracks often develop in the seal and portions of the samples can be lost through these cracks.

(3) Do not fill the bulb more than two-thirds full when the sample is very volatile. The bulb may burst as it comes to room temperature.

(4) The face of the worker should be shielded when he is handling sealed ampoules which contain liquids boiling below 20°C, since faulty ampoules may not be able to bear the pressure.

This technique was found very satisfactory in analyzing methyl vinyl ether (b.p. 5 to 6°C), ethylene oxide (b.p. 10.7°C), ethyl amine (b.p. 17°C), and acetaldehyde (b.p. 21°C). Fuming sulfuric acid samples were also measured by this technique.

The bulb size depends on the size of sample needed. The use of 5-mm outside diameter tubing in stage 1 (Fig. 27) will yield bulbs about 1/8 to 3/16 inch in diameter; these bulbs will hold about 0.05 to 0.2 g. The use of 7-mm tubing will yield bulbs that range from 1/4 to 1/2 inch in diameter and will hold about 0.2 to 0.7 g. When 9- and 12-mm tubing is used, the bulbs will be 3/4 inch to 1 inch in diameter and will hold about 1 to 3 g. For the very low-boiling samples, 15°C or below, the ampoules should be blown small so that the walls remain rather heavy. The ampoule should be tested for its ability to hold the pressure. A simple test is to tap the bulb sharply on a wooden surface, when it is in stage 4 (Fig. 27); the ampoule is held by the end of the stem. If the bulb withstands this test it will hold a sample boiling down to 0°C. (During the test, the bulb should be turned slightly between taps to make sure there are no weak spots.)

When gases such as ethylene oxide, butadiene, or methyl vinyl ether are sampled at room temperature, the cylinder containing

the sample should be cooled well below the boiling point of the sample. The cylinder is then inverted and the sample is *poured* in the liquid state into a tall bottle which is immersed about one-half its length in crushed dry ice. The bottle should be capped while it is cooling in the ice to prevent condensation of water on the inside. Also, it should be capped immediately after the sample is in to prevent carbon dioxide from dissolving in the sample (this is sometimes very significant). The sampling of the material in the liquid state insures obtaining a representative sample from the cylinder. The samples are then drawn from the bottle into ampoules, as described above.

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